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THE ENDOGENOUS DEVELOPMENT OF THE COCCIDIA OF THE FERRET, AND THE HISTOPATHOLOGICAL REACTION OF THE INFECTED INTESTINAL VILLI

BY

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(Received for publication 1 *February*, 1935)

The coccidia of the ferret were first described about eight years ago (Hoare, 1927). It was shown that this animal harboured three distinct species, *Isospora laidlawi*, *Eimeria ictidea* and *E. furonis*, and the account given of the exogenous stages of development of these parasites provided all the data of systematic value.*

In the case of the two species of *Eimeria*, experimental infections were produced in two ferrets by feeding them on large numbers of mature oöcysts. These were obtained from the faeces of naturally infected animals and were preserved in a solution of chromic acid, in which their sporulation took place. Before proceeding with the experiments it was established by daily examination of the stools for one month that the two ferrets in question were free of coccidial infection.

After the infective meal, the ferrets were kept under daily observation till oöcysts first appeared in their faeces ; they were then sacrificed and the entire intestines were fixed in an alcoholic solution of Bouin's picro-formol and preserved in 70 per cent. alcohol.

The present account of the endogenous development of *Eimeria ictidea* and *E. furonis* is based mainly on sections of the intestines of the experimental ferrets, though material obtained from ferrets with natural ' pure ' infections was also used to some extent.†

The sections were stained with Mayer's acid haemalum and counterstained with aqueous eosin.

As regards the third coccidium, *Isospora laidlawi*, it was not possible to study its development satisfactorily owing to the extreme scantiness of the infection in the ferrets examined by me.

The endogenous cycle of both species of *Eimeria* follows the conventional lines, and would not warrant a detailed description if it were not for the fact

*Since in some publications I am credited with describing the coccidia of an '*African* ' ferret, I take this opportunity to point out that both the polecat, *Mustela* (*Putorius*) *putorius*, and its domesticated variety, the ferret, *M.* (*P.*) *p.* var. *furo*, are limited in their geographical distribution to Europe. As stated in my earlier paper, the ferrets which served for the investigation were all obtained in England.

†Some of this material was kindly placed at my disposal by Dr. P. P. Laidlaw, F.R.S., to whom my thanks are due.

that some of the stages provide differential diagnostic characters which supplement those already noted in the exogenous stages of development of these coccidia (Hoare, 1927). Moreover, further interest is added by the fact that *E. ictidea* provokes a peculiar histological reaction on the part of the infected intestinal villi of its host.

EIMERIA FURONIS Hoare, 1927

The development of *E. furonis* takes place in the epithelium of the small intestine and rectum of the ferret. The distribution of the parasites in the mucous membrane is very irregular : in some sections large areas are seen to be densely packed with them, while others are quite free from infection. In the small intestine the parasites may invade the entire length of the villi as far as the opening to Lieberkühn's crypts, but they are mainly concentrated in the apical portion, their numbers rapidly diminishing towards the crypts. In the rectum the coccidia are restricted to the epithelium of the free surface of the mucous membrane, viz., to the ridges between the openings of the glands of Lieberkühn.

Within the epithelial cells the parasites occupy a position between the nucleus and the lumen of the gut. The infections observed were fairly heavy ones, the host-cells in the more densely invaded portions of the epithelium usually harbouring several parasites, the number present being limited only by the accommodation available.

Though the experimentally infected ferret received a single dose of mature oöcysts of *E. furonis*, it would seem that their endogenous development did not proceed *pari passu*, since various stages of development up to the formation of oöcysts were well represented.

Similar conditions were observed in the intestines of the naturally infected ferrets, the only difference being in the relative proportion of the various forms of the parasite present. Thus, in the experimental ferret the stages of schizogony were predominant, while in the naturally infected animals the sexual forms were more numerous. The various stages are indiscriminately mixed and do not exhibit an arrangement into ' age-groups,' as is the case with *E. ictidea*.

The earliest stages (as seen in the experimental ferret) were spherical forms, about 3–4μ in diameter, with a relatively bulky nucleus consisting of a deeply staining karyosome and an unstained outer area (fig. A, 1). This form obviously represents the trophozoite or undivided young schizont. By successive nuclear divisions and growth this form develops into the ripe schizont (fig. A, 2, 3, 4), from the surface of which the merozoites are budded off, leaving a cytoplasmic residue (fig. A, 5). The merozoites produced by these schizonts are stumpy, sausage-shaped bodies measuring about 3–4μ in length and 2μ in breadth (fig. A, 6).

In addition to the type of schizogony just described, another was observed which results in the production of a different form of merozoites. These

are elongated curved bodies, measuring about 6μ by $1{\cdot}3\mu$, with one end of the body rounded and the other drawn out. A compact nucleus is situated near the blunt extremity (fig. A, 7, 8).

Schizogony of the second type was encountered almost exclusively in the naturally infected ferrets, in which the sexual forms of the parasite were predominant. In these cases small and medium-sized ovoid forms (fig. A, 9, 10), evidently representing young gametocytes, are present in large numbers. The elongated merozoites (fig. A, 8) can easily be linked up through these forms with the fully developed gametocytes (fig. A, 11, 12). It would thus appear that the first type of schizogony (fig. A, 1–6) represents the asexual reproductive cycle of *E. furonis*, while the second type results in the formation of sexually differentiated merozoites or gamonts which give rise to the gametocytes.

The macrogametocyte (or macrogamete) is spherical, about 8μ in diameter, and contains darkly staining globular inclusions of reserve material (fig. A, 11). The male gametocytes and gametes (fig. A, 12, 13) present no special peculiarities. When examined fresh the oöcyst of *E. furonis* measures on the average about

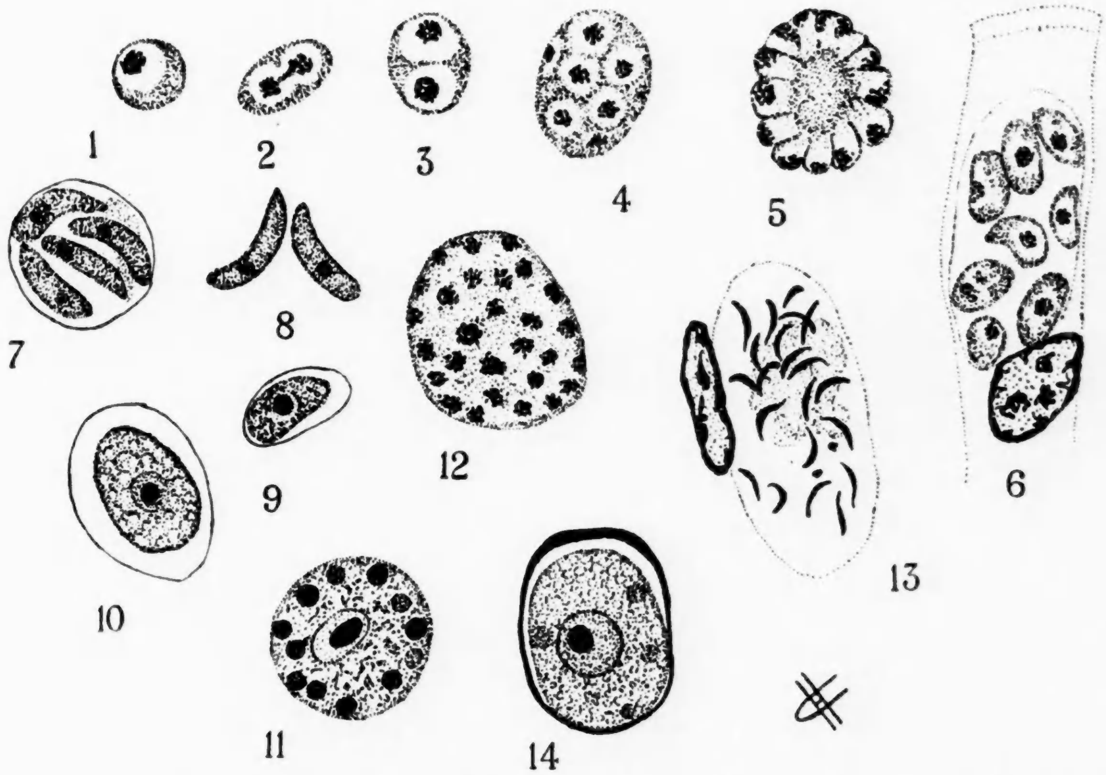

FIG. A. *Eimeria furonis.* 1-7.—Schizogony: 1.—Young schizont or trophozoite; 2, 3.—First nuclear division; 4.—Later stage of schizogony; 5.—Merozoites budding off from the surface of schizont; 6.—Schizogony completed: host-cell vacuole containing fully developed merozoites of agametogenous generation; 7.—Stage equivalent to 6, but producing merozoites of gametogenous generation. 8.—Merozoites of gametogenous generation free in lumen of gut. 9, 10.—Young gametocytes. 11.—Macro-gametocyte, or gamete, with globular inclusions. 12.—Microgametocyte in process of gamete-formation. 13.—Fully developed microgametes and gametocytic residue in vacuole of host-cell. 14.—Oöcyst with zygote (wall crumpled owing to fixation). (All the stages depicted, except in 8, were intracellular. Drawn with the aid of a camera lucida at × 2,000.)

$12{\cdot}8\mu$ by $12{\cdot}0\mu$, the zygote being about $9{\cdot}6\mu$ in diameter. In sections, as is to be expected, the dimensions are reduced, while the oöcyst wall is usually somewhat crumpled as the result of fixation (fig. A, 14).

The infection with *E. furonis* exerts no harmful effect upon the host, while the mucous membrane of the intestine does not exhibit any special histological reaction to the parasitic invasion. The only effect observed is an enlargement and irregular arrangement of the epithelial cells in the most heavily infected parts of the mucous membrane and the denudation of some areas due to the shedding of infected epithelium, as is usual in coccidiosis.

EIMERIA ICTIDEA Hoare, 1927

The endogenous development of *E. ictidea* proceeds in the epithelium of the small intestine of the ferret. The infections studied were extremely heavy, but the distribution of the parasites in the mucous membrane was ' patchy,' the villi in some regions being packed with them, while in others they were entirely free from infection.

Unlike *E. furonis*, *E. ictidea* invades only the free portion of the villi, especially the tip, the epithelial cells never harbouring more than one parasite, although in the early stages of development, when the parasite is as small as the forms of *E. furonis*, there is room for more than one. However, as the parasite grows it gradually fills up the entire available space in the host-cell, the nucleus of which is shifted towards the base of the cell. This early adaptation obviously provides for the future requirements of the growing parasite.

The development of *E. ictidea* appears to proceed in a more regular manner than that of *E. furonis*, for the great majority of forms observed in the experimentally infected ferret were represented by the sexually differentiated stages, ranging from the young gametocytes to the fully developed gametes and oöcysts. The only indubitable stage of schizogony seen was the final one with fully formed merozoites. It would thus appear that all the sporozoites of *E. ictidea* introduced in a single dose develop more or less simultaneously, and at the same rate. This is one of the reasons why certain stages of development, viz., those of early schizogony, were not observed.

Another characteristic feature of *E. ictidea* is the arrangement of the parasites in ' age-groups ' or ' colonies.' While in *E. furonis* all stages are found freely intermingled in the epithelium, in *E. ictidea* forms belonging to the same stage are grouped together. In some cases a villus is occupied only by a colony of fully developed schizonts, or of mature male and female gametocytes, or of young forms, etc., while in other cases a group of forms representing one stage may be contiguous to an independent group of another stage.

The youngest forms observed were merozoites (fig. B, 2) lying free in the lumen of the gut in the neighbourhood of groups of fully segmented intracellular schizonts (fig. B, 1) from which they had arisen. The merozoites are elongated vermicular bodies measuring about 11μ by 1μ, with one extremity

rounded and the other drawn out to a point. The compact nucleus is situated near the rounded extremity. After penetrating into the host-cell, the merozoites shorten (fig. B, 3, 4), become rounded and grow in size (fig. B, 5, 6). These forms measure from 3μ to 4μ in length or diameter. They evidently represent

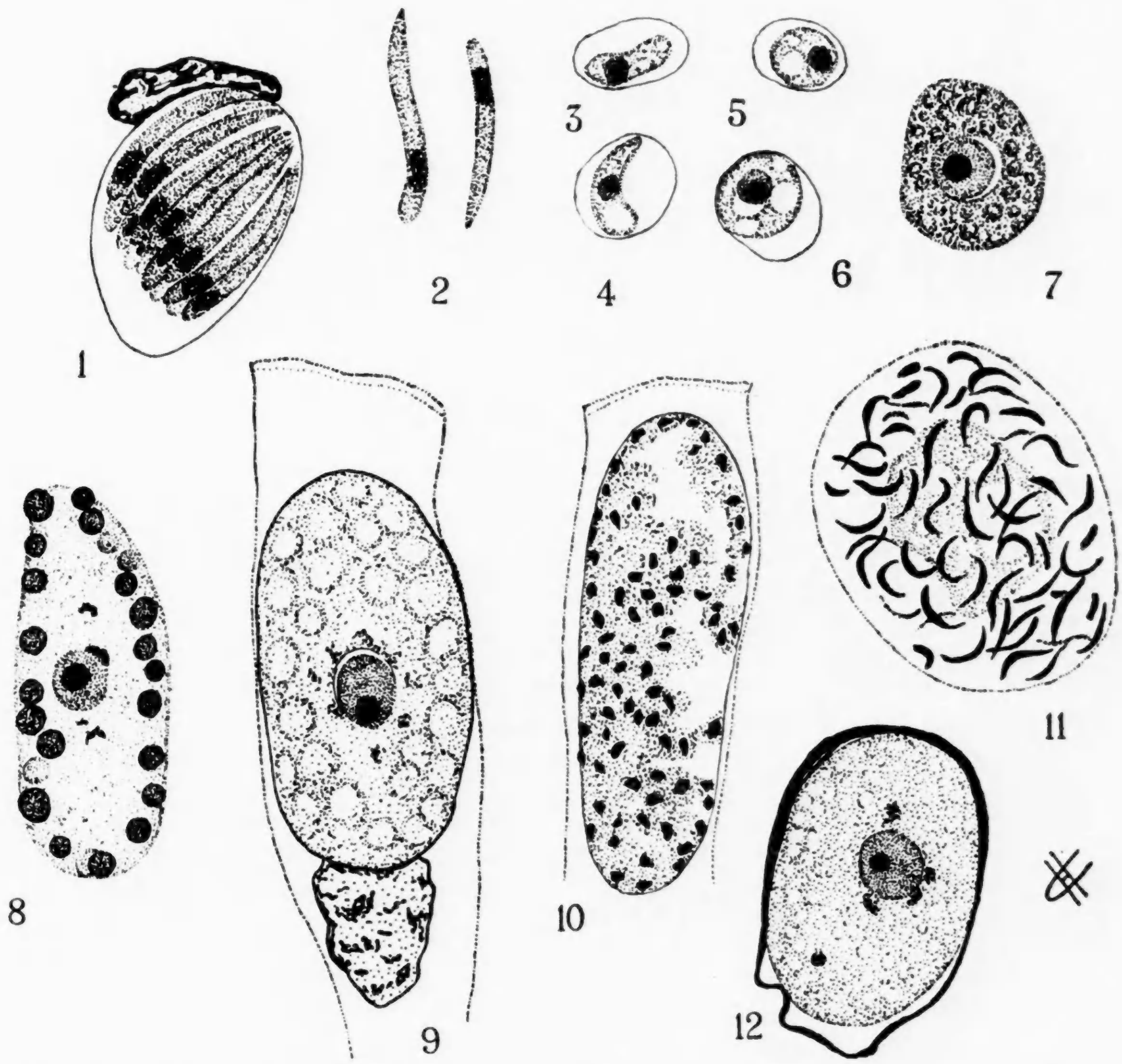

FIG. B. *Eimeria ictidea.* 1.—Final stage of schizogony, showing group of merozoites enclosed in vacuole of host-cell (only the nucleus of latter is drawn). 2.—Two merozoites free in the lumen of the gut. 3-6.—First stages of development of gametocytes (shown within vacuole of host-cell) : 3, 4.—Earliest forms—merozoites in the process of shortening and rounding ; 5, 6.—Small rounded gametocytes. 7.—Young macrogametocyte. 8.—Mature macrogametocyte containing peripheral globular inclusions. 9.—Macrogamete with cyst-wall in process of formation at the expense of the globular inclusions. 10.—Microgametocyte in process of gamete-formation. 11.—Fully developed microgametes and gametocytic residue. 12.—Oöcyst with zygote (the cyst-wall is crumpled as the result of fixation). (All the stages depicted, except the free merozoites (2), were intracellular. Drawn with the aid of a camera lucida at $\times$ 2,000.)

young gametocytes, since on the one hand there is a gradual transition from them to the unmistakable gametocytes (fig. B, 7), and on the other hand none of these or the larger forms showed any signs of nuclear division suggestive of schizogony (cf. *E. furonis,* fig. A, 2, 3).

The stages of gametogony were fully represented. In the young macrogametocyte (fig. B, 7), which measures about 9μ by 7μ, the cytoplasm is packed with granular matter which is later transformed into the dark-staining globules of reserve material characteristic of the fully developed female gametocyte. The latter form is always elongated, measuring about 20μ by 7μ, and occupies the entire enlarged host-cell (fig. B, 8). In the macrogamete or early zygote the cytoplasm is full of vacuoles, the dark reserve material previously contained in them having been used up for the formation of the oöcyst wall (fig. B, 9).

The average size of the oöcyst when discharged with the faeces is about $23{\cdot}6\mu$ by $17{\cdot}5\mu$, the zygote measuring about 15μ by 12μ. The appearance of the oöcyst in sections is shown in fig. B, 12. In all the stages of macrogamete formation the nucleus has the characteristic vesicular structure. The male gametocytes and gametes (fig. B, 10, 11) differ from the corresponding stages of *E. furonis* only in their larger dimensions.

HISTOLOGICAL REACTION PRODUCED BY *E. ICTIDEA*

In an earlier paper (Hoare, 1927) it was noted that the ferrets parasitized by coccidia manifested no outward signs of the infection and that no macroscopic lesions or alterations could be detected in the intestinal mucosa.

In the course of the microscopic examination of sections in connection with the present work it was found that, while infection with *Eimeria furonis* produced no special histological changes (cf. p. 114), in the case of *E. ictidea* there is a very marked tissue reaction of an unusual type.

As already stated, these coccidia invade only the free end of the villus, sometimes only its summit, while the basal portion, usually the greater part of the villus, is uninfected. When the epithelium harbours only the small young forms of the parasites (trophozoites and young gametocytes) the villus retains its normal appearance (fig. C, 1, and Pl. I, A) and does not seem to be altered in any way. However, when the extremity of the villus harbours the large old forms of the coccidium (schizonts, gametocytes, oöcysts) there is evident a tissue reaction which tends to isolate the infected portion from the non-infected one.* This takes the form of an annular constriction which separates the two portions (*cn* in fig. C and Pl. I). It involves the epithelium and extends into the core of the villus to a depth varying with the degree of its development. Apparently in its earliest phase the constriction represents only a slight circular furrow in the epithelium, not deeper than the height of the epithelial cells (*cn* in fig. C, 2 ; Pl. I, B, C) ; later, however, it increases in depth (Pl. I, D, *cn*), penetrating more and more into the core, till the villus assumes an irregular hour-glass shape with a more or less narrow ' waist ' (fig. C, 3, 4 ; Pl. I, E, F).

The parasitized and normal epithelium forming the upper and lower

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*Though the small forms never by themselves call forth the reaction, they are occasionally drawn into the isolated infected area together with the large forms (cf. Pl. I, G).

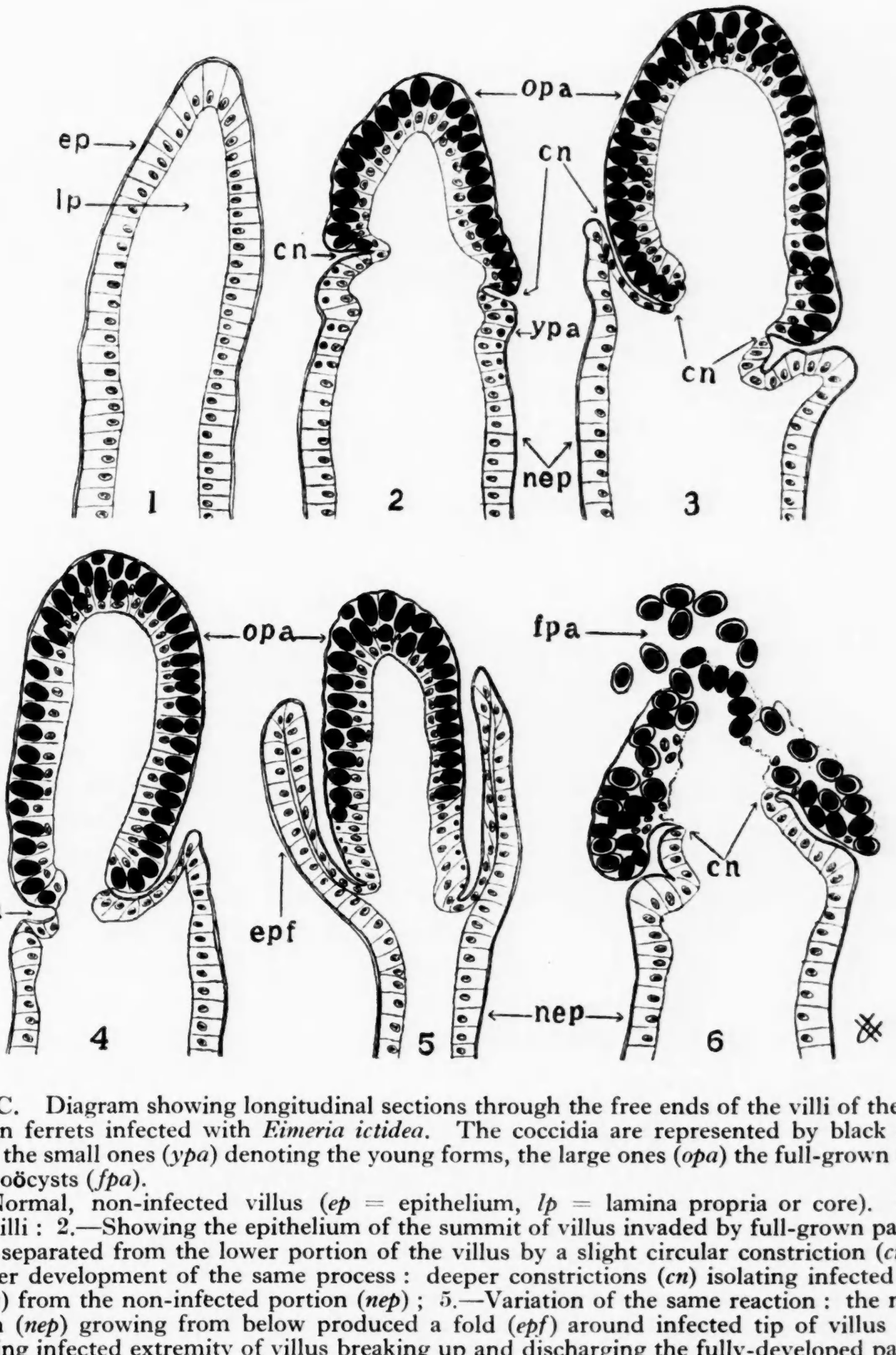


FIG. C. Diagram showing longitudinal sections through the free ends of the villi of the small intestine in ferrets infected with *Eimeria ictidea*. The coccidia are represented by black circles and ovals, the small ones (*ypa*) denoting the young forms, the large ones (*opa*) the full-grown forms, including oöcysts (*fpa*).

1.—Normal, non-infected villus (*ep* = epithelium, *lp* = lamina propria or core). 2-6.—Infected villi : 2.—Showing the epithelium of the summit of villus invaded by full-grown parasites (*opa*) and separated from the lower portion of the villus by a slight circular constriction (*cn*) ; 3, 4.—Further development of the same process : deeper constrictions (*cn*) isolating infected tip of villus (*opa*) from the non-infected portion (*nep*) ; 5.—Variation of the same reaction : the normal epithelium (*nep*) growing from below produced a fold (*epf*) around infected tip of villus (*opa*) ; 6.—Showing infected extremity of villus breaking up and discharging the fully-developed parasites (*fpa*) into the lumen of the gut. (The figures were outlined with the help of a camera lucida, some of them corresponding to sections depicted in Plate I, viz., 1=A, 2=B, 3=F, 5=G, 6=H. For abbreviations see explanation of Plate I.)

surfaces respectively of the annular constriction face each other and meet at the bottom of the groove. As the constriction deepens the contact between the infected and non-infected epithelium—which forms a continuous layer—is reduced to a thin circular line, seen as a point in section (fig. C, 3, 4, *cn*), so that the infected extremity is supported practically only by the core of the villus.

The infected portions of the villus also show the following pathological changes. The blood-vessels in the lamina propria are dilated and closely packed with erythrocytes (Pl. I, B, *cap*), and in a number of instances extravasation of these elements into the surrounding tissue is evident. Moreover, in the majority of the villi the affected portions manifest various degrees of necrosis (Pl. I, F).

As far as I could ascertain, the reaction just described appears to have no parallel in any other known pathological condition. The formation of the constriction is undoubtedly due mainly to the activity of the sound epithelium of the villus, and the following would appear to be the most plausible interpretation of the processes involved.

It is known that under normal conditions there is a constant desquamation of portions of the epithelium which are being replaced by new cells arising from the crypts of Lieberkühn, the regenerative process being particularly active at the summit of the villi, where the oldest epithelial cells are situated. A similar reparation usually takes place in the case of coccidial infections in which destruction of the epithelium is brought about by the discharge of the oöcysts into the lumen of the gut. Whatever the cause of the local shedding of epithelium may be, compensation is effected by the new epithelial tissue gradually pushing its way over and closing the gap. Under the conditions mentioned above, regenerative proliferation of the epithelium does not take place until its denudation sets in.

The histological reaction observed in infections with *E. ictidea* would appear to represent a modification of the usual type of epithelial regeneration. Since in the infected summit of the villus all the epithelial cells are fully occupied by the large forms of the parasite, it is obvious that the whole of this portion of the epithelium ceases to perform its normal functions and that, from the physiological point of view, it represents a dead, inactive part of the villus. Apparently this condition serves to stimulate the proliferative activity of the epithelial cells at the base of the villus in the same way as a local destruction of the epithelium does, with the result that new epithelial cells are pushed upwards. However, instead of progressing unimpeded, as in cases where there is a gap to be covered, the further advance of the new epithelium is prevented by the inert mass of infected epithelium at the summit of the villus. On reaching this obstruction, the proliferating epithelium, unable to advance, grows inwards, thereby forming a constriction of increasing depth around the core of the villus. Owing to the tension exerted by the active new epithelium at the point where it meets the parasitized epithelium, the contact between them may be reduced to a mere circular line (fig. C, 3, 4, *cn*), and, since this is obviously the weakest point of

the epithelial layer, it is easily ruptured here, as shown in Pl. I, I, in which the sound epithelium is disconnected from the infected epithelium and forms a collar-like extension (*epc*) surrounding the summit of the villus. Of course, it is impossible to ascertain from sections whether the rupture and the collar-like projection of the epithelium were brought about by an active process, viz., by the proliferating epithelium actively breaking away from the parasitized epithelium and growing upwards, or whether they were due to post-mortem contraction of the villus under the influence of fixation, in the course of which the epithelium is ruptured at the constriction and the infected tip drawn inwards leaving the newly formed epithelium behind. Whatever the cause of the phenomenon, it demonstrates clearly the ease with which the connection between the infected and non-infected epithelium may be severed.

The same mechanical reasons which lead to the formation of the constriction cause the growing epithelium in this region to be thrown into an annular fold, which frequently develops to such an extent that it surrounds the infected summit of the villus in the form of a cup or sheath (*epf* in fig. C, 5, and Pl. I, G).

The pathological changes observed in the lamina propria of the infected portion of the villus can be attributed to the pressure exerted by the annular epithelial constriction, probably combined with the pressure of the enlarged parasitized cells, while it is conceivable that the parasites themselves may also have some harmful effect upon the tissues. The pressure exerted by the constriction would account for the dilatation of the capillaries (Pl. I, B, *cap*), leading to their congestion with red blood corpuscles and the extravasation of these, and for the resulting focal necrosis of the infected tip of the villus (Pl. I, F), which assumes the character of a sequestrum.

None of the processes, whether degenerative or regenerative, taking place in the infected villus have the slightest effect upon the vitality of the coccidia, which continue their normal development. The usual method by which the mature parasites reach the outer world is by the necrosis of the summit of the villus and the discharge of the coccidial oöcysts (and incidentally other stages) into the lumen of the intestine (*fpa* in fig. C, 6, and Pl. I, H). It is also possible that the infected extremities of the villi may be bodily dislodged from their slender supports (cf. Pl. I, E, F, and fig. C, 3, 4) by the movements of the intestine and its contents.

As regards the significance of the histopathological reaction described, it would appear to be primarily reparative, the formation of constrictions and folds reflecting the obstacles standing in the way of the growing epithelium. The regenerative process can evidently be successfully completed in villi in which the parasites together with the broken up (or intact) extremities are cast off (fig. C, 6 ; Pl. I, H). When this occurs there is no further hindrance to the new epithelium covering up the gap in the normal way. It is doubtful if this reaction can be regarded as of protective importance from the point of view of the host, since it does not check the development of the parasites. However, it is possible

that a certain diminution in the number of parasites may take place when the tips of the villi containing immature coccidia break off and are carried away with the faeces.

As is known, the effect produced by coccidia upon their hosts varies considerably, ranging from the local destruction of the intestinal epithelium—which usually has no harmful effect upon the host—to extensive inflammation and necrosis of large areas of the mucosa, accompanied by haemorrhage and other marked pathological symptoms, frequently leading to the death of the host.

As far as it was possible to ascertain from the literature on coccidioses, the peculiar reaction observed in the ferret is unique. It seems probable, however, that similar phenomena are produced by other coccidia, but have been overlooked, since in cases in which no macroscopic lesions are evident and the health of the host is not impaired, the minute microscopic alterations of the tissues produced by the parasites are usually ignored, as being of no practical importance.

In infections with closely related coccidia, especially in those which parasitize the same host, belong to the same genus and differ from each other mainly in quantitative characters, one is frequently struck by the great difference in the effect they produce upon the host. Excellent examples of this are afforded by the six species of *Eimeria* parasitic in domestic fowl (Tyzzer, 1929, and Tyzzer, Theiler and Jones, 1932) and by the two species of the same genus in the ferret. But whereas the fowl-coccidia differ in their localization, some being intra-epithelial, some sub-epithelial, and others in both situations, the localization of the ferret-coccidia is uniform. They both invade the epithelium, and, though the distribution of *E. furonis* in the villus is diffuse, both species are concentrated in masses at the summit, each epithelial cell of which is fully occupied by one (*E. ictidea*) or more parasites (*E. furonis*). The localization and density of the infection being more or less the same in both these parasites, it would appear that the difference in their effect upon the host is due to some difference in their physiological activities.

SUMMARY

A description is given of the endogenous stages of development of the two species of *Eimeria* parasitic in the ferret, *Mustela* (*Putorius*) *putorius* var. *furo*, and of their effect upon the host.

Both coccidia develop exclusively within the epithelial cells of the intestinal mucosa.

E. furonis invades the small intestine and rectum, while *E. ictidea* is found in the small intestine, being localized in the summit of the villi.

E. furonis has no special effect upon the host-tissues, while *E. ictidea* calls forth a peculiar histopathological reaction, viz., the formation of a deep constriction separating the infected summit of the villus from its basal portion.

REFERENCES

HOARE, C. A. (1927). On the coccidia of the ferret. *Ann. Trop. Med. & Parasitol.*, **21**, 313.
TYZZER, E. E. (1929). Coccidiosis in gallinaceous birds. [I.] *Amer. Jl. Hyg.*, **10**, 269.
——— THEILER, H., and JONES, E. E. (1932). Coccidiosis in gallinaceous birds. II: A comparative study of species of *Eimeria* of the chicken. *Ibid.*, **15**, 319.

EXPLANATION OF PLATE I

Photomicrographs of longitudinal sections through the free ends of villi of the small intestine in ferrets infected with *Eimeria ictidea* (× *ca.* 60). (Cf. text-fig. C.)

Abbreviations: *cap* = capillaries in core of villus ; *cn* = constriction around villus ; *ep* = epithelium of villus ; *epc* = epithelial ' collar ' ; *epf* = epithelial fold ; *fpa* = free (discharged) parasites (oöcysts and gametocytes) ; *lp* = lamina propria or core of villus ; *nep* = normal (non-infected) epithelium of villus ; *opa* = old parasites (chiefly gametocytes) within epithelium ; *ypa* = young parasites within epithelium.

A. Normal, non-infected villus.

B. Infected villus : the epithelium of the summit, harbouring large forms of the parasite (*opa*), is separated from the lower portion of the villus, containing small parasites (*ypa*), by a slight circular constriction (*cn*) ; the capillaries (*cap*) within the core of the villus are dilated and densely crowded with erythrocytes.

C. Infected villus : heavily parasitized summit separated from non-infected lower part by well-defined constriction (*cn*).

D. Villus in which a large portion of the free end is infected and the constriction (*cn*) separating it from the non-infected portion is consequently produced at a lower level.

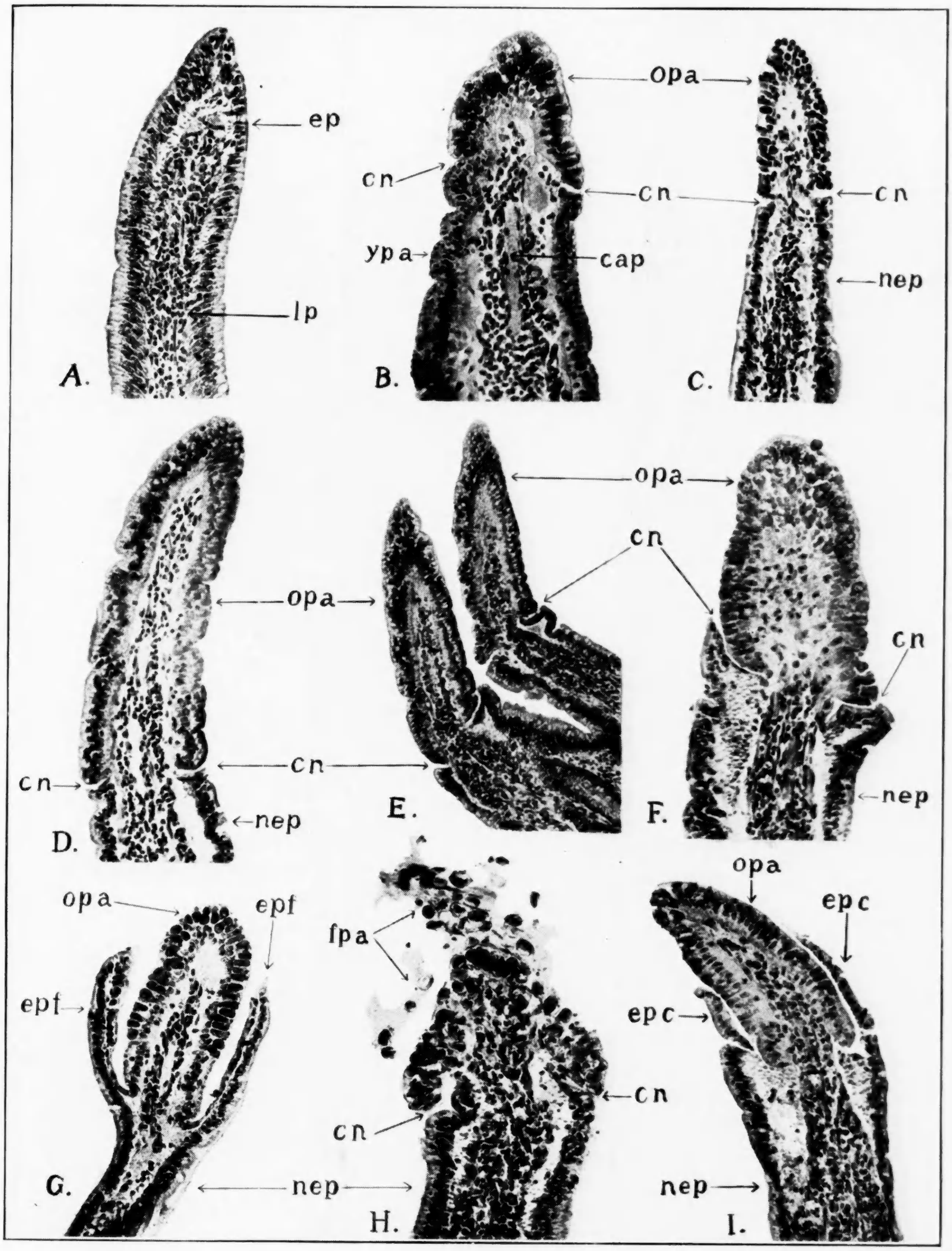
E. Villi with further development of the tissue reaction : deep constrictions (*cn*) separate the infected extremities from the non-infected basal parts of two villi.

F. Villus with very deep constriction (*cn*) between infected tip of villus and non-infected basal portion ; the invaded part is necrotic.

G. Villus in which the proliferating normal epithelium (*nep*) has produced a fold in the form of a cup (*epf*) surrounding the infected extremity (*opa*) ; the latter contains some young parasites in addition to full-grown ones.

H. Villus with extremity breaking up and the parasites (*fpa*) being discharged into the lumen of the gut.

I. Villus in which the proliferating normal epithelium (*nep*) is not continuous with the infected epithelium (*opa*), but forms a collar-like extension (*epc*) around the infected tip of the villus, which is supported only by the lamina propria.



H. R. Grubb, Ltd., Poplar Walk, Croydon

THE DIAGNOSTIC VALUE OF LEISHMANIA VACCINE

BY

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(Received for publication 31 *January*, 1935)

A diagnosis of cutaneous leishmaniasis based on the clinical appearance of the lesion, the microscopical findings and, if necessary, the histological picture

As a rule, the clinical signs are sufficient for the diagnosis of cutaneous leishmaniasis, but the finding of the parasites is sometimes essential to confirm that diagnosis.

In the initial stage of the disease, L.D. bodies are usually numerous, but their number decreases gradually as the lesions become chronic, particularly in the tuberculoid type of lesion. In histological preparations of chronic lesions either no parasites are found, or they may be present in small numbers. Bettmann and Wasielewski (1909) have also noticed that out of 49 cases only 37 gave positive microscopical findings. Out of 70 cases considered in the present paper, 57, or 77 per cent., were positive by examination of smears. It is very difficult to find the parasites in cases where the lesion is infected with pyogenic organisms or where energetic treatment for ulcers was instituted through mis-diagnosis. The material for examination is to be taken from the periphery of the lesion where the infiltrated area has not yet reached the stage of ulceration or infection, and repeated examinations may be necessary. When the material is properly taken, tissue cells as well as blood cells should be visible in the stained preparation. In case of repeated negative findings we attempted to diagnose by histological examination of the lesions. Even though this examination does not always give a specific picture, nevertheless it helps to confirm the diagnosis. Moreover, owing to negative findings both in smears and histological preparations, in a number of cases an additional diagnostic test was made.

It appeared that a disease which confers immunity might exhibit allergic manifestations in the skin. In 1926, Professor Adler, of the Hebrew University, at our request kindly prepared a vaccine for experimental purposes. The results of the first set of experiments in the treatment of cutaneous leishmaniasis by means of vaccine were indefinite (Dostrovsky, 1929). At first there were very severe reactions after vaccination, which resulted in sterile abscesses in the skin. (This was most likely due to residues of medium in the material injected.) It was necessary to prepare a vaccine as free as possible from liquid culture medium used for cultivation of parasites and, in addition, to determine a minimal dose for intradermal injection, which would be powerful enough to

cause a skin reaction.* Following Professor Adler, the vaccine was prepared as follows :—

1. Culture on Locke serum agar semi-solid medium.

2. When growth appears, remove the top layer and free it from the culture medium by triple washing with saline solution and centrifuging.

3. Mix the washed parasite with saline solution and determine the number of parasites in 1 c.cm. in a haemocytometer.

4. Add phenol and saline until a 0·5 per cent. concentration of phenol and the number of parasites desired per 1 c.cm. is obtained.

It is possible that in spite of repeated washings traces of culture medium may adhere to the parasites ; but this is of no great significance, since the specific action of the vaccine is quite clear.

The total number of cases examined by the Leishmania vaccine reaction was 70. This reaction will be designated as L.V.R., i.e., Leishmania vaccine reaction. An urtica was formed intradermally by injecting 0·1 c.cm. of a suspension containing one million parasites per c.cm. At a distance of 4 cm. below the urtica, another injection was given for control, consisting of 0·5 per cent. phenol in saline. The reaction was read 24 to 48 hours later. At the site of the injection there appeared an area of erythema, varying in size in different individuals. In the centre of each area there was a considerable degree of infiltration in the positive cases (see Pl. II), but in the negative ones there was either a sclerotic point which could be palpated only at the site of the puncture, or no manifestations at all. The following case is an illustration of the changes caused by the L.V.R. and the accompanying histological reactions.

A specimen for histological study taken from a six-day-old infiltration area following an inoculation with Leishmania vaccine intradermally

Epidermis—akantosis, hyperkeratosis.

Cutis—The papillary layer was partly invaded by many invading cells ; the papillary aspect had almost disappeared. The infiltration which was located mostly in the perivascular area contained cells poor in protoplasm with nuclei full of chromatic material (lymphocytes) and many cells with elongated nuclei (histiocytes). No parasites were found. The dead parasites disappeared from the lesion within six days after the injection (probably digested by the invading cells).

All cases that manifested a skin reaction of 0·5 cm. and more were considered positive, and only three cases, i.e., 4 per cent., were found negative. In three patients the reaction appeared only after the second inoculation. The three negative cases all had ulcers, in which L.D. bodies were found on microscopical examination ; unfortunately, no re-vaccination was done.

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*Professor Adler believes that the use of Locke serum instead of Locke serum agar will result in an inoculum free from culture medium.

A dose of 100,000 parasites (0·1 suspension of 1,000,000 Leishmania parasites in 1 c.cm.) seems to be the minimal dose necessary for sensitization. In some cases negative results were manifested with a dose of 0·05 c.cm., and only when this was raised to 0·1 c.cm. was the reaction positive. The reaction persisted in many cases for a week and more. In many cases, where the first microscopical examination revealed no parasite, the L.V.R. was positive and repeated examinations eventually gave positive results. The following case is instructive.

A patient had four boils of three months' duration. The clinical diagnosis was Leishmania nodosa, but no parasites were found. Since the L.V.R. gave a positive reaction (erythema and an infiltration of 1·0 cm.), we continued to search for the parasite, and only after taking the fourth specimen for examination were L.D. bodies found.

The size of the erythema varied from 0·5 to 7 cm. in diameter. The strength of the reaction is divided into three degrees :—

(*a*) Erythema plus infiltration of 0·5 and less than 1·0 cm. in diameter will be indicated by +.

(*b*) Erythema plus infiltration of 1·0 cm. by + +.

(*c*) Erythema plus infiltration above 1·0 by + + +.

The severity of the reaction does not depend upon the number of boils. One case of Leishmania nodosa with four lesions showed a reaction of 1·0 cm. Another case with only one ulcerated lesion gave a reaction of 7 cm. The chronicity of the lesion also does not affect the severity of the reaction. Thus a 3 cm. reaction was noted in two cases of about six weeks' duration, of which one was classified as Leishmania nodosa and the other as Leishmania ulcerata. On the other hand, the reaction was slightly more than 0·5 cm. in a case of Leishmania nodosa, which, according to the patient, was present for eleven months. In another case of Leishmania nodosa, which was present for two years, the reaction was only 1·0 cm. in diameter.

Interesting relationships exist between the type of clinical manifestations and the severity of the L.V.R. These are more noticeable when the boils are classified into two types, the nodal and the tubero-ulcerating.

Severity of reaction	Leishmania nodosa		Leishmania tuberco-ulcerosa	
+	8	36 per cent.	2	6 per cent.
++	10	46 ,,	10	29 ,,
+++	4	18 ,,	22	65 ,,

The degree of the reaction and its relation to clinical findings was studied in 56 cases. In spite of the fact that this number is small, it is nevertheless

interesting that out of 10 cases with a reaction of +, only 2 cases were of the tubero-ulcerative type, the rest (8) being of the nodal type. Out of 26 cases with a reaction of + + +, 4 cases are classified under the nodal type and 22 cases under the tubero-ulcerative type.

The local reaction in cutaneous leishmaniasis may be compared to those found in syphilis and tuberculosis. In the tuberco-ulcerative types of lues and tuberculosis there is marked sensitization and an intense skin reaction, as in the corresponding type of cutaneous leishmaniasis.

The sensitivity of the skin remains unaltered for a long time and can be provoked many years after cure. Thus positive reactions were found in 10 adult cases who in childhood had suffered from Leishmania lesions which had left disfiguring scars on the face.

A positive reaction was also obtained in human experimental leishmaniasis. Thus a case infected in the Department of Parasitology of the Hebrew University in 1925 and cured spontaneously in 1927 was inoculated with vaccine on December 30th, 1929, and gave a positive reaction which persisted until January 15th, 1930.

Montenegro (1926) obtained results similar to ours : out of 37 cases of cutaneous Leishmania only 5 were negative. (This author used extracts of culture free from parasites.)

The L.V.R. is more sensitive, for there were only 3 negatives out of 70 cases.

The value of the reaction depends not only on the positive results, which approach 100 per cent. in cutaneous leishmaniasis, but in the negative reactions obtained in healthy individuals or those affected with other diseases.

Seventy cases suffering from skin and other diseases were tested for L.V.R. A positive reaction was seen in 5 cases of lepra mixta, one case of psoriasis, and one case of sycosis trichophytica. The degree of reaction in these cases did not exceed +.

The following Table (page 127) gives the distribution of positive reaction in individuals affected with various diseases.

The non-specificity of the vaccine is 10 per cent. if we include reaction indicated by +. It is necessary to test a larger number of individuals in the case of each disease in order to arrive at definite conclusions, especially so in those diseases which show a positive + or a doubtful + (?) reaction.

Though the non-specificity is only 10 per cent., the differential diagnosis is not difficult. This fact enables us to attribute great importance to the L.V.R. for the diagnosis of cutaneous leishmaniasis, especially so in those cases where microscopical examination fails.*

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*Professor Adler informed us that the reaction is of little or no value for the diagnosis of Mediterranean visceral leishmaniasis.

CONCLUSIONS

Seventy cases of Leishmania were tested for L.V.R. by intradermal inoculations of dead organisms of L. tropica, and 98 per cent. gave positive reactions. The reaction was considered positive if within 48 hours it reached a minimal size of 0·5 cm. in diameter (erythema and infiltration). The L.V.R. was performed by the injection of 0·1 c.cm. of an emulsion containing one million parasites in 1 c.cm. of 0·5 per cent. solution of phenol. The control consisted of 0·5 per cent. solution of phenol in physiological salt solution injected three to four finger-breadths below the site of the inoculation with L.V.R. in the arm.

Disease	Negative	Less than	+	++	+++
Pyodermia	14	1	—	—	—
Trichophytia	1	—	—	—	—
Actinomycosis	1	—	—	—	—
Urethritis gonorrhoeica	—	1	—	—	—
Tuberculosis colliquativa	4	—	—	—	—
Erysipelas	1	1	—	—	—
Epidermophyton inguinale	2	—	—	—	—
Abscessus idroticus	1	—	—	—	—
Acne conglobata	1	—	—	—	—
Acne vulgaris...	3	—	—	—	—
Myositis ossificans	1	—	—	—	—
Cancroid	—	1	—	—	—
Eczema	1	2	—	—	—
Sterilitas	1	—	—	—	—
Stomatisis aphtosa	1	—	—	—	—
Psoriasis	2	—	1	—	—
Lues latens	—	1	—	—	—
Pernio ulcerosa	3	—	—	—	—
Ulcus cruris varicosum	3	—	—	—	—
Mastitis	1	—	—	—	—
Alopecia areata	3	1	—	—	—
Ischias...	1	—	—	—	—
Lepra mixta	1	—	5	—	—
Combustio	1	—	—	—	—
Sycosis trichophytica profunda ...	—	—	1	—	—
Tuberculosis papulonecrotica ...	2	—	—	—	—
Tuberculosis indurativa	1	—	—	—	—
Pityriasis rosea	1	—	—	—	—
Condylomata acuminata	1	—	—	—	—
Verruca	3	—	—	—	—
Total	55	8	7	0	0

The largest reaction was obtained in cases of Leishmania tubero-ulcerosa. Out of 70 cases not affected with Leishmania, there was a positive reaction (plus 1) in 7 cases, i.e., 10 per cent.

I am indebted to Dr. Gurevitz, of the Laboratory Department of the Hadassah Hospital, to Dr. S. Kaplan, of the Rothschild Hospital, and to Professor Adler, of the Hebrew University, for their help.

REFERENCES

BETTMANN and WASIELEWSKI (1909). Zur Kenntnis der Orientbeule und ihres Erregers. *Arch. f. Schiffs- u. Trop.- Hyg.*, **13**, Bhft. 5, 175.

DOSTROVSKY, A. (1929). Zur Behandlung der Leishmaniosis cutanea. *Ibid.*, **33**, 417.

MONTENEGRO, J. (1926). Cutaneous reaction in leishmaniasis. *Arch. Derm. & Syph.*, **13**, 187.

PLATE II

EXPLANATION OF PLATE II

LEISHMANIA RECIDIVA NODOSA

L.V.R. test over the left arm (after 48 hours)

H. R. Grubb, Ltd., Poplar Walk, Croydon

STUDIES ON THE FACTORS THAT MAY INFLUENCE THE TRANSMISSION OF THE POLYMORPHIC TRYPANOSOMES BY TSETSE

IX.—ON THE INFECTIVITY TO *GLOSSINA* OF THE TRYPANOSOME IN THE BLOOD OF THE MAMMAL

BY

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In 1912, Robertson working at Mpumu in Uganda studied for the first time the development of a member of the *brucei* group of trypanosomes in mammalian blood and attempted to correlate the various phases of this development with the power of the parasite to survive in its natural vector, the tsetse. The career of the trypanosome in the fly she called the exogenous cycle ; the changes in the vertebrate, the endogenous cycle (Robertson, 1913).

Her work led to the following conclusions : that the trypanosome in the vertebrate shows a continuous range of variation in form ; that the shorter forms are those destined to carry on the cycle in the transmitting host ; and that there are in the course of the endogenous cycle definite periods when the parasites in the blood of the vertebrate are not infective to fly, although trypanosomes may be present in large numbers.

Since this early work no one has produced any serious experimental study on this subject, although the importance of the principle enunciated by Robertson has been admitted from time to time by people concerned with the transmission of trypanosomes by tsetse.

At the Second International Conference on Sleeping Sickness convened in Paris in 1928, a research programme was adopted which included the investigation of this particular subject ; and ever since the appointment of the League of Nations Commission in Uganda in 1926 the infectivity of the trypanosomes of man to tsetse has been an essential part of our regular research programme in Uganda. In the Final Report of the Commission the expression ' transmissibility ' was adopted to describe what appears to me to be a definite quality of the tsetse-carried trypanosomes. The many figures published in connection with various transmission experiments carried out at Entebbe all have a bearing on the endogenous cycle, and in the present paper a selection is made from these extensive records to illustrate what appear to be different phases of this phenomenon.

Robertson believed that, ' Given reasonably favourable conditions of temperature and moisture, it is the strain of trypanosomes and not the fly that within a relatively wide range plays the deciding *rôle* in limiting the number of infected glossina.' (Robertson, 1912.)

In my own earlier work I adopted as a working hypothesis the assumption that there are certain forms of the trypanosome in the mammal specially fitted to infect tsetse, and that when a fly takes up a sufficient number of these forms that fly becomes infected. This hypothesis took no account of the differences between one individual fly and another, nor, incidentally, between one race or one species and another.

The experience of recent years has shown that the subject is by no means so simple as this. The part played by the fly, both species and individual, is undoubtedly of considerable importance, and there is evidence that the temperature also exerts an influence (Taylor, 1932).

Part of the evidence submitted in this paper has been selected from the records of some 15 years of experimental study of the transmission of many different strains of polymorphic trypanosomes. During that time certain impressions have been acquired about the laws governing the transmission of these organisms by tsetse. Some of these have been set forth in previous publications. For example, it has been shown, I hope conclusively, that there exist in nature in Africa strains of trypanosomes which at the time of their original isolation from the vertebrate, man or antelope, are non-transmissible by *G. palpalis* (Duke, 1930). Some of the strains of this category were examined over a prolonged period of maintenance at the laboratory in a number of different mammalian hosts, and remained consistently non-transmissible. Some were tested in *G. morsitans* and found equally impotent in that species of tsetse. Again, the disappearance of transmissibility has been witnessed in a number of strains of *T. gambiense* during prolonged upkeep at the laboratory, strains that for some time after their original isolation were readily transmissible by *G. palpalis*. These observations all point to an inherent and permanent change in the attributes of the trypanosome itself, quite unconnected with the variability of its insect vector.

It is, I think, certain that strains of trypanosomes differ in their ability to infect tsetse. As a rule *T. rhodesiense* is more readily transmissible by tsetse than is *T. gambiense*, an explanation being that *T. rhodesiense* is normally dependent on the game tsetses (relatively good transmitters) in nature.

As stated elsewhere, I believe that the instability of the quality transmissibility—in the case of *T. gambiense* this character is easily impaired and even lost—suggests that the association between tsetse and these trypanosomes is of relatively recent origin, the adjustment between them being still very imperfect (Duke, 1930). The investigator is thus confronted with a protozoon at best but imperfectly adapted to its insect transmitter, which in turn varies greatly in its suitability to the requirements of the trypanosome. The combinations that result are of an almost infinite variety.

The fact that the *G. palpalis* of the Victoria Nyanza, the species with which most of the Uganda work has been carried out, is undoubtedly a relatively poor transmitter, renders experimental proof of the cycle all the more difficult to attain.

It may be objected that a ' strain ' of trypanosomes such as those isolated and studied at Entebbe has no counterpart in nature, where mixture of strains and of species must be constantly occurring in every fly-belt. With the trypanosomes of man this admixture must also occur, though probably not to the same extent as with the species non-pathogenic to man. But the study of isolated strains is the only way of reaching an understanding of the biology of these trypanosomes, and once this has been done the effects of re-infection and superinfection can easily be studied, together with the important questions of immunity therewith connected (Duke, 1931).

As to the particular problem before us at the moment, it will be seen that the experiments actually devised to demonstrate the operation of the endogenous cycle have failed in their object. But their failure is, I feel sure, due to the inadequacy of their conception, there being so many variable factors that nothing short of an exhaustive investigation with a large number of flies can hope to succeed.

A selection of the experiments performed is given below, in the hope that they may be of assistance to those who are in a position to carry on the investigation of this interesting and, one might venture to say, important subject.

In what follows, experiment = a box of some 50 laboratory-bred flies ; g. = *T. gambiense* ; rh. = *T. rhodesiense* ; Damba = the Damba trypanosome ; p. = guinea-pig ; m. = monkey ; pal. = *G. palpalis* ; mor. = *G. morsitans*. Except where *G. morsitans* is expressly mentioned, all the flies used were *G. palpalis*.

Series I (Table I)

Paired experiments, the two components being fed at the same time on the same infecting animal. Thereafter, one of the 2 boxes is nourished on a clean animal, whereas the other is fed again on one, two or three more occasions on an infected animal.

Thus, taking the first pair of experiments of the Table, nos. 133 and 134 : experiment 134 was fed once on the infected pig 22A and then had no more feeds on trypanosome-containing blood ; but the flies of experiment 133 on the 14th, 16th and 18th days after their single original feed on p. 22A were fed on m. 793 (infected with *T. gambiense*). Experiment 133 thus had in all four feeds on an infected animal, as against experiment 134's one feed.

In Table I, under the main heading ' Secondary Infecting Feeds,' the subhead ' Day of Commencement ' shows the number of days that elapsed between the original infecting feed and the commencement of the secondary exposures. These secondary feeds on an infected animal were consecutive, with an interval of 48 hours' starvation between them, until the requisite number was completed.

It will be noted that the secondary infector was either the one used for the original feeding or a different animal carrying a different strain and sometimes a different species of trypanosome ; ' *stat* ' in column 5 means that the secondary

feeds followed immediately the original feed at 48-hourly intervals, the same infector being used throughout.

In the first 10 pairs of experiments of this Table an interval of at least 12 days elapsed before the secondary feeds began, and any infected flies dying within this period obviously derived their flagellates from the original infecting feed. Moreover, allowing a minimum of 16–18 days for a fully developed gland infection in the conditions of experiment at Entebbe, a number of the gland infections in flies dissected during these experiments were definitely attributable also to the original infector—hence the last column of the table, the figures in which may of course *all* relate to the original infection.

TABLE I

Experiment	Original infecting feed		Secondary infecting feeds				Number of flies dissected	Positive flies	
	Animal	Trypanosome	No.	Day of commencement	Animal	Trypanosome		Total	Infections possibly attributable to secondary feeds
133	P22A	rh.	3	14th	793	g.	55	1	0
134	P22A	rh.					49	1	
140	M802	rh.	2	16th	811	rh.	37	2	0
141	M802	rh.					34	0	
143	M801	rh.	4	12th	795	g.	55	4	0
144	M801	rh.					48	3	
145	M802	rh.	3	14th	793	g.	44	0	
146	M802	rh.					40	1	
149	M801	rh.	3	22nd	{810, 811}	rh.	60	2	0
150	M801	rh.					25	0	
154	P22A	rh.	3	20th	{810, 811}	rh.	46	3	0
155	P22A	rh.					31	1	
158	M801	rh.	3	13th	{810, 811}	rh.	54	1	
159	M801	rh.					51	0	
160	P25A	rh.	3	12th	810	rh.	48	0	
161	P25A	rh.					57	0	
164	M802	rh.	3	12th	810	rh.	55	5	4
165	M802	rh.					35	2	

TABLE I—(*continued*)

Experiment	Original infecting feed		Secondary infecting feeds				Number of flies dissected	Positive flies	
	Animal	Trypanosome	No.	Day of commencement	Animal	Trypanosome		Total	Infections possibly attributable to secondary feeds
167	M802	rh.	3	12th	810	rh.	42	3	1
168	M802	rh.					41	0	
172	M802	rh.	3	8th	810	rh.	45	2	2
173	M802	rh.					49	1	
193	M793	g.	4	*stat*	793	g.	56	2	
194	M793	g.					59	0	
225	M776	g.	4	8th	776	g.	38	4	3
226	M776	g.					40	2	
230	M776	g.	4	*stat*	776	g.	34	3	
231	M776	g.					50	3	
244	M789	rh.	4	*stat*	789	rh.	35	6	
245	M789	rh.					50	4	
247	M789	rh.	3	*stat*	789	rh.	37	5	
248	M789	rh.					39	3	
254	P22B	rh.	3	*stat*	22B	rh.	44	1	
255	P22B	rh.					50	3	
256	M776	g.	4	*stat*	776	g.	43	7	
257	M776	g.					38	0	
258	P22C	rh.	4	*stat*	22C	rh.	56	2	
259	P22C	rh.					45	3	
260	P22B	rh.	4	*stat*	22B	rh.	32	1	
261	P22B	rh.					21	2	
262	776	g.	4	*stat*	776	g.	44	0	
263	776	g.					47	6	
264	820	rh.	3	*stat*	820	rh.	45	5	
265	820	rh.					46	2	
266	820	rh.	3	*stat*	820	rh.	47	3	
267	820	rh.					46	2	
270	P22B	rh.	2	*stat*	22B	rh.	46	4	
271	P22B	rh.					40	3	
272	M776	g.	3	*stat*	776	g.	44	0	
273	M776	g.					49	2	

In 16 of the 25 pairs the multiple feed experiment has more infected flies than its companion, but in 5 of these 16 pairs it is certain that all the infected flies were infected at the original and not during the secondary feeds. In 6 of the pairs the experiment that fed only once had more infected flies than the other. The Table indicates, therefore, a slightly greater number of infections in the boxes that had the secondary infecting feeds.

SERIES II (Table II)

Each of the tests of this series consists of 4 or 5 experiments, instead of a pair as in Series I. The flies of one experiment of each test fed on 3 or 4 different days on the infecting animal, and on each of these days a box of clean flies fed on the same animal once only. Here the results are even less definite than in Table I. They certainly indicate no superiority of multiple over the single feeds.

The experiments of Series I and II were devised on the assumption that the infectivity to fly of the trypanosomes in the blood varies from day to day, so that in the long run flies which feed on the infected animal on several occasions will have a better chance of becoming infected than those feeding only once.

Numerous experimental records show clearly that the periods during which the blood is infective to fly extend often over a number of consecutive days, whereas the negative periods of a transmissible strain usually appear to be of short duration. It is evident, therefore, that the chances are in favour of a test falling in a positive period, during each day of which the trypanosomes are, according to the theory we are investigating, in a state ' infective ' to fly.

In such circumstances all the control (single-feed) flies will take up infective trypanosomes, although possibly to a different degree on different days, and variations between individual flies, together with what Robertson styles the general inhibiting capacity of the *Glossina* (which in *G. palpalis* is relatively high), will largely determine the results. Ideally, these tests should encompass both a ' positive ' and a ' negative ' period, beginning, say, during a negative phase, so that some of the controls will feed during a negative phase. To attain this end, very much more extensive tests must be employed.

The figures in Tables I and II do, however, make possible a comparison between the results of single and of multiple feeds on infected blood, and to this end an analysis by Mr. J. S. McDonald, Laboratory Assistant to the Institute, is given below.

' In the combined experiments the single feed contained 2,353 flies, with 96 (4·08 per cent.) positive, and the multiple feed boxes contained 1,701 flies, of which 81 (4·76 per cent.) were positive.

' The multiple feed-boxes in Series I contained only 10 positive flies in which the infection could be possibly attributed to secondary feeds, and if *all* the positive flies in Series II are admitted as deriving their infection from secondary feeds the total for the combined Series is only 25.

TABLE II

Experiment	Trypanosome	Number of feeds on infecting animal	Number of flies	
			Dissected	Infected
1436	rh.	3	109	1
1438	,,	1	54	3
1444	,,	1	44	1
1449	,,	1	51	1
1503	rh.	3	106	2
1505	,,	1	46	2
1512	,,	1	45	0
1520	,,	1	50	0
1441	rh.	3	94	2
1443	,,	1	51	3
1446	,,	1	49	1
1450	,,	1	50	7
1018	rh.	4	81	2
1020	,,	1	37	0
1025	,,	1	69	1
1032	,,	1	59	2
1040	,,	1	54	1
229	g.	3	35	0
230	,,	1	27	4
234–6	,,	1	69	3
241	,,	1	44	4
251	g.	3	37	4
252	,,	1	57	7
254	,,	1	32	0
258	,,	1	37	1
348	rh.	4	53	2
349	,,	1	38	5
353	,,	1	53	0
357	,,	1	50	0
367	,,	1	27	2
350	rh.	4	44	2
351	,,	1	44	2
358	,,	1	49	0
366	,,	1	43	1
370	,,	1	44	1

'The infection rate due to the primary feeds is 3·76 per cent. (at least), and the difference between this and the rate for all the multiple feed-boxes is well within the limits of variations encountered in transmission experiments with any one strain.

'There is no evidence in the data examined that multiple feeding results in a higher infection rate than does single feeding, and comparison by means of the χ^2 formula reveals no significant difference between the results obtained in the single feed and multiple feed experiments.

'After corrections for statistical errors are applied to the observed percentages of infection, there is no suggestion, even at a probability of 1 to 1, of any increase due to multiple feeding.'

TABLE III, which represents another attempt at raising the infection rate of laboratory-bred *G. palpalis* by repeated exposure to the chance of infection, confirms this conclusion.

TABLE III

Experiment	Infected animal	Number of feeds	Number of flies	
			Dissected	Positive
155	488 (g.)	11	50	1
161	491 (g.)	5	58	1
206	509 (rh.)	5	17	1
263	503 (g.)	5	93	1
264	514 (g.)	9	39	1
266	516 (g.)	9	44	3
271	503, 516 & 574 (g.)	8	31	1
276	522 (rh.) & 516 (g.)	6	31	1

In each of the first 6 experiments the same infecting animal served for all the feeds.

In experiment 271 three different monkeys, all carrying *T. gambiense*, were used ; and in experiment 276 two were used, one infected with *T. rhodesiense* and the other with *T. gambiense*.

SERIES IV comprises figures from transmissibility tests selected from the records of the Institute, to illustrate various phenomena of common occurrence.

In perusing the figures of this section the following points should be borne in mind :—

(1) A preliminary feed or two by newly hatched flies on clean blood before they feed on an infected animal makes no apparent difference to the eventual number of infected flies found in dissection.

TABLE IV

	Trypanosome	Experiment number	Date	Number of flies	
				Dissected	Positive
A. Consistent infectivity	XLIII rh.	57	29.10.30	107	2
		64	30.10.30	115	19
		79	4.11.30	124	8
		98	10.11.30	114	12
		104	12.11.30	91	7
		109	14.11.30	93	11
		111	15.11.30	82	10
	XLIV rh.	59	29.10.30	106	13
		67	31.10.30	122	16
		77	4.11.30	146	8
		86	7.11.30	108	8
		91	8.11.30	104	11
	Tinde II rh.	954 mor.	14. 9.32	46	4
		955 ,,	,,	40	2
		958 ,,	15. 9.32	38	1
		959 ,,	,,	52	7
		970 ,,	18 & 19. 9.32	32	8
		971 ,,	,,	37	1
B. Periods of intensified infectivity	XXIV Damba	197	20 & 22. 9.26	57	1
		208	26 & 28. 9.26	42	1
		221	30.9 & 1.10.26	47	3
		230	4, 5 & 6.10.26	56	13
		242	7, 8 & 10.10.26	72	14
	XXIV Damba	196	20 & 22. 9.26	55	2
		204	24 & 25. 9.26	39	1
		210	27 & 28. 9.26	46	2
		231	4 & 6.10.26	64	14
		237	5 & 7.10.26	35	8
	XX rh.	164	13. 8.26	50	0
		165	14 & 16. 8.26	45	3
		175	19 & 21. 8.26	39	6
C. Non-infective periods	XXIV Damba	146	28 & 30. 7.26	41	4
		149	29 & 30. 7.26	48	8
		152	31.7 & 1. 8.26	49	9
		156	2 & 3. 8.26	48	11
		157	4 & 6. 8.26	48	0
		161	7 & 9. 8.26	51	0
	Tinde I rh.	626	26 & 28. 4.32	50	10
		627	27 & 29. 4.32	49	6
		629	29.4 & 1. 5.32	51	0
		630	30.4 & 2. 5.32	37	0

TABLE IV—(*continued*)

	Trypanosome	Experiment number	Date	Number of flies	
				Dissected	Positive
C. (*con.*) Non-infective periods	LV brucei	923 mor.	8 & 9. 9.32	28	2
		924 „	„	39	5
		931 „	11 & 12. 9.32	39	0
		932 pal.	„	51	0
		945 mor.	13 & 14. 9.32	31	4
		946 pal.	„	49	2
	Tinde II rh.	1610	19. 8.33	206	0
		1619	4. 9.33	93	1
		1632	12. 9.33	89	5
		1637	13. 9.33	106	5
		1639	14. 9.33	55	0
		1646	16. 9.33	67	0
	Tinde II rh.	1699	25 & 26. 9.33	34	1
		1700	26. 9.33	44	1
		1709	27. 9.33	49	2
		1713	28. 9.33	42	0
		1719	29. 9.33	43	0
		1725	30. 9.33	42	0
		1730	1.10.33	50	0
		1733	2.10.33	45	0
	Tinde III rh.	1740	4.10.33	28	1
		1744	5.10.33	45	0
		1748	6.10.33	45	0
		1752	7.10.33	53	0
		1758	8.10.33	42	2
		1760	9 & 10.10.33	42	2
		1763	10 & 11.10.33	52	2
		1766	12 & 13.10.33	49	4
		1767	13 & 15.10.33	41	1
		1770	14 & 15.10.33	57	4
	Tinde III rh.	1915	8 & 10. 2.34	82	3
		1917	8 & 11. 2.34	86	2
		1920	10 & 12. 2.34	39	1
		1926	12 & 14. 2.34	50	0
		1933	14 & 16. 2.34	43	0
		1941	16 & 10. 2.34	53	0
		1945	17 & 19. 2.34	41	0
		1946	19 & 21. 2.34	44	0
		1950	21 & 23. 2.34	71	0
		1953	22 & 24. 2.34	82	0

(2) All the *G. palpalis* used in these experiments came from the same pupa grounds, so that there is no question of racial difference in the transmitting power of the flies of different batches.

(3) The meteorological conditions under which these investigations were carried out may be regarded as constant.

Data regarding strains non-infective to and non-transmissible by *G. palpalis* have already been published (Duke, 1930).

Section A illustrates consistent infectivity of the trypanosome to tsetse.

Section B illustrates periods of intensified infectivity.

Section C illustrates non-infective periods.

Section D illustrates the results of feeding flies *on man* for several consecutive days, a fresh box of flies being fed each day. All these patients were infected (naturally) with *T. gambiense*, and the flies were put on before the commencement of any treatment.

Table IV gives the figures for section A, B and C.

Of these three sections, C is the most instructive, showing that on certain occasions, although trypanosomes may be numerous in the blood, they are non-infective to tsetse. It is, I think, safe to assume that the non-infectivity of these negative periods is absolute, although the numbers of flies hitherto employed in these tests hardly justifies a final opinion.

With non-transmissible strains there is now a considerable amount of evidence that the inability to use the fly is indeed absolute. According to my experience, once this power is lost it is never recovered, save of course by admixture with another transmissible strain.

Table V illustrates section D. All the flies had their infecting feeds on the untreated patient. The strain numbers in all these Tables are those of the Institute series.

CONCLUSIONS

1. A study of the Entebbe records of transmission experiments with man's trypanosomes and *G. palpalis* lends support to Robertson's views on the endogenous cycle expressed in 1912.

Of special interest is the evidence of the existence of 'negative phases' in the development of the trypanosome in the mammal, phases during which the trypanosomes though often numerous in the blood are non-infective to the tsetse.

2. An examination of the experimental section of this paper suggests that repeated feeds on an infected animal during a positive phase of the cycle do not increase the infection rate of the flies over that produced by one such feed.

This paper is the last of the present series. The prevailing motif of these nine studies is academic, a word which loomed large and ominous in the deliberations that marked the earlier years of trypanosomiasis research in Uganda. This admission therefore implies a certain betrayal of responsibility. At every turn our programme has been subjected to the closest scrutiny by jealous guardians of the public interest, and at times the very existence of the Institute has hung on the issue, academic or utilitarian.

TABLE V

Strain	Experiment number	Date of infecting feeds	Number of flies	
			Dissected	Positive
IV	57	26. 4.26	37	3
	58	27. 4.26	39	1
	60	28. 4.26	40	2
	61	29. 4.26	39	0
VII	95	24. 6.26	45	3
	96	25. 6.26	45	1
	99	28. 6.26	45	3
	101	29. 6.26	45	6
VIII	97	27. 6.26	46	0
	98	28. 6.26	56	0
	100	29. 6.26	36	0
	102	30. 6.26	45	0
IX	141	23. 7.26	49	5
	142	24. 7.26	61	5
	143	25. 7.26	48	6
XII	321	27.12.26	58	3
	325	28 & 29.12.26	53	0
	329	30 & 31.12.26	46	0
XIII	322	27.12.26	49	0
	326	28 & 29.12.26	47	0
	331	30 & 31,12,26	43	3
XIV	323	27.12.26	43	3
	328	28 & 29.12.26	46	1
	332	31.12.26 & 1.1.27	46	3
XV	324	27.12.26	58	3
	327	28 & 29.12.26	45	3
	330	30 & 31.12.26	51	1
XVI	400	10.11.26	59	0
	401	11 & 13.11.26	34	2
	403	12 & 14.11.26	46	3
	406	15 & 17.11.26	48	1
XVIII	495	24 & 26. 5.27	42	0
	496	25 & 27. 5.27	44	0
	503	28 & 30. 5.27	50	0
	505	29 & 31. 5.27	46	0
XIX	211	28. 9.26	42	0
	219	29. 9.26	46	0
	222	1.10.26	47	0
	223	2.10.26	40	0

'The research programme proposed concerns, moreover, study of the trypanosome rather than of human trypanosomiasis. The danger of such investigations becoming merely academic need not be further stressed.' So reads one of the later and more enigmatical of these critical pronouncements. It should therefore be understood that a great deal of the evidence put forward in these studies was acquired, not in *ad hoc* experiments, but in the course of years of investigations of unimpeachable utility along a number of different lines. Moreover, although it has been intended for years past to broach these interesting topics, their treatment has been postponed until it became obvious that the career of the Institute was drawing to a close. Now, with but a few months to run, I feel that there is no further need for restraint, and indeed it appears to be a duty to expose all available information, however obscure its value may be at the moment of publication.

REFERENCES

DUKE, H. L. (1930). On the occurrence in man of strains of *T. gambiense* non-transmissible cyclically by *G. palpalis*. *Parasitol.*, **22**, 490.

——— (1931). *Trypanosoma gambiense* in monkeys and ruminants; prolonged infection, immunity and superinfection. *Ibid.*, **23**, 325.

ROBERTSON, MURIEL. (1912). Notes on certain aspects of the development of *Trypanosoma gambiense* in *Glossina palpalis*. *Rep. Sleeping Sickness Comm. Roy. Soc.*, XII, 138.

——— (1913). Notes on the life-history of *Trypanosoma gambiense*, with a brief reference to the cycles of *Trypanosoma nanum* and *Trypanosoma pecorum* in *Glossina palpalis*. *Ibid.*, XIII, 119.

TAYLOR, A. W. (1932). The development of West African strains of *Trypanosoma gambiense* in *Glossina tachinoides* under normal laboratory conditions, and at raised temperatures. *Parasitol.*, **24**, 401.

SUSCEPTIBILITY AND RESISTANCE TO A TRYPANOSOME INFECTION

IX.—ACTIVE IMMUNIZATION OF RATS AND GUINEA-PIGS AND PASSIVE IMMUNIZATION OF RATS TO A TRYPANOSOME INFECTION

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In recent years the mechanism of resistance to trypanosome infections has engaged the interest of a number of investigators. Of special importance are the extensive studies of Taliaferro and his associates. The problem is important in itself, but is of special interest because a clear understanding of the mode of defence to this parasite may also throw light on the defensive mechanism in other protozoan blood infections, more particularly in malaria in man and babesia in cattle.

An important consideration in the study of trypanosome immunity is the difference in the degree of resistance manifested by different animals and by the same animals at different ages. In the mouse, the process of development often proceeds in geometrical progression as in a favourable test-tube culture (Doerr and Berger, 1922). In the rat, destruction and multiplication proceed simultaneously, and can be followed from the moment trypanosomes appear in the circulation until the end (Kligler and Comaroff, 1929). During this entire period no circulating antibody can be demonstrated, either by *in vitro* or *in vivo* test (Kligler, 1931). On the other hand, our observations, as well as those of Andrews, Johnson and Dormal (1930), have shown that the terminal stages of the infection are characterized by an exhaustion of the reticulo-endothelial system both in the liver and spleen. This is further shown by the fact that splenectomized rats possess a decidedly lower resistance to infection than normal ones, the course approximating to that in the mouse (Kligler, 1929).

In guinea-pigs and cats the infection assumes a more chronic character. During the early stages of the infection there is destruction of the trypanosomes without evidence of antibody in the circulation. After a time, varying with each animal, demonstrable lytic antibodies appear in the circulation, but the time of appearance and concentration of the lytic antibody is in no apparent way related to the number of trypanosomes in the circulation or to the periods of their invasion prior to the demonstrable presence of antibody (Kligler, 1931). After

varying intervals relapses occur, and the animals may succumb at a time when the blood is apparently free of parasites. In guinea-pigs, therefore, both cellular and humoral activity are demonstrable, the active elaboration of humoral antibodies accounting for the low grade relapsing character of the infection. The relapses are presumably due primarily to the successive selection of serum-resistant variants which are no longer sensitive to the lytic antibody. This selection can be repeated *in vitro* and a variety of strains obtained (Kligler, 1931). The importance of the cellular factor in the guinea-pig is, however, indicated by the fact that blocking the reticular system by the injection of olive oil, or by some other means, results in relapse (Kligler and Weitzman, 1924).

In the experiments reported below we have approached the problem of resistance from two different angles. On the one hand, we have attempted to ascertain whether immunity can be produced in rats, guinea-pigs and rabbits by repeated injection of dead trypanosomes. On the other hand, we have studied the possibility of passive immunization of rats by the injection of guinea-pig-, rabbit- or cat-serum containing demonstrable trypanolytic antibodies.

EXPERIMENTAL

1. *Active Immunization of Rats*

In this series of experiments we attempted to ascertain the effect of repeated injections of suspensions of dead trypanosomes on the resistance of rats to a subsequent infection with the same strain.

The dead trypanosome suspensions were prepared in the following manner :—Rats were bled into citrate solution when the infection was at its height, and the trypanosomes were separated from the red cells by fractional centrifugation. The red cells were sedimented first by slow centrifugation for five minutes and the trypanosome suspension in the supernatant serum was removed. The red cells were then resuspended in saline to the original volume and again sedimented, many of the previously sedimented trypanosomes now remaining in the saline suspension. The saline and serum suspension were then mixed and centrifuged at high speed for half an hour to an hour. The supernatant fluid was discarded and the packed trypanosomes were resuspended in a volume of saline equal to twice the original blood volume. This suspension was placed at 37° C. for one hour, and then overnight in the ice box. After this period the organisms as a rule were all dead and could be used as vaccine.

A number of experiments were made to determine the effect of repeated injections of trypanosomes. Daily injections of 2 c.cm. were given. Some groups received 10, others 20, injections. At various intervals after the completion of the treatment, the rats were infected i.p. with the rat-passage strain, and the course of the infection was followed. In a number of rats the trypanocidal power of the serum was tested before the infection. The results of these experiments are summarized in Table I.

Eight experiments were carried out, including a total of 91 treated and 51 control rats. It is apparent from the data (Table I) that 10 to 20 daily injections of dead trypanosomes considerably increased the resistance of the treated rats. The route or number of injections did not appreciably affect the results ; while there were some variations in the individual experiments, the results were remarkably consistent.

TABLE I

Effect of repeated treatment of rats with suspensions of dead trypanosomes on their resistance to infection with the same strain

Date injection started	No. of injections	No. of rats in group		Average weight in gm.	No. of days between treatment and infection	Infective* dose	Average incubation time ; in days	Duration of infection ; in days	
		i.v.	i.p.					Mean	Median
5.3.33	20		5		15	15,000	4·4	10·0	10·0
Control	0		9			15,000	2·1	7·5	7·0
4.6.33	20	3		67·0	1	8,000	4·0	12·0	
	20		8	59·0	1	8,000	4·0	10·6	10·0
	10		8	57·4	1	8,000	4·5	12·5	13·0
Control	0		7	59·3		8,000	2·3	7·2	6·5
9.7.33	10	4		81·0	3	8,000	5·0	11·4	11·5
	10		4	81·0	3	8,000	5·0	12·0	12·5
Control	0		4	86·0		8,000	2·3	9·5	9·5
6.8.33	10	10		50·0	1	10,000	6·8	12·4	12·5
	10		9	49·0	1	10,000	7·0	12·7	14·0
Control	0		9	44·0		10,000	2·1	6·8	7·0
25.8.33	10	7		75·6	7	8,000	5·1	13·5	14·5
	10		7	76·6	7	8,000	5·0	13·7	13·5
Control	0		7	78·0		8,000	3·3	7·6	6·5
21.12.33	10		9	218·7	3	7,000	5·3	11·7	13·0
Control	0		5	212·6		7,000	2·8	7·8	8·0
21.12.33	10		10	47·3	3	7,000	8·3	15·5	16·5
Control	0		5	44·6		7,000	3·2	8·6	7·0
Trypanosomes	10		7	212·0	4	13,000	7·0	16·1	
Saline	10		6	194·0		13,000	2·5	8·5	
Control	0		5	202·0		13,000	2·0	7·6	

*The rat-passage strain was used throughout these experiments.

It is noteworthy that the most striking difference is in the incubation time, which is twice as long in the treated as in the untreated rats. If each experiment is taken as a unit and the results averaged, it works out that the mean incubation time for treated groups is 5·4 days, and for the untreated groups 2·7 days, whereas the difference between the mean incubation period and the mean total duration of illness is 7·4 days in the treated groups and 5·7 days in the control groups. Calculated on the basis of the total number of rats, the results are practically the same. The 91 treated rats had an average duration of illness of 13·4 days, while that of the 51 controls was 7·6 days, a difference of 5·8 days ; the mean incubation periods of the two groups were 5·8 and 2·5 days respectively ; deducting these periods from the respective total duration of illness, 13·4 and 7·6 days, it works out that the total duration of active infection is 7·6 and 5·1 days. In other words, the difference between the mean incubation periods of the two groups is over 100 per cent., while that between the duration of active infection is only 50 per cent.

Obviously the increased resistance of the treated animals manifested itself primarily in the early stages of the infection, before the number of parasites had become sufficiently large to invade the peripheral circulation. Once that occurred, the progress of the infection was retarded to a much lesser extent. This would suggest that the principal effect of the treatment is to stimulate the activity of the cellular elements. The subsequent experiments were designed to ascertain the character of the reaction responsible for the increased resistance.

It was naturally of interest to determine whether the blood of the immunized rats contained specific antibodies. Blood drawn from rats which had received 10 to 20 injections was tested *in vitro* by the method previously described (Kligler, 1931). The results were irregular. Out of 10 rat-sera tested, 2 showed some lytic properties in high concentration, while the rest were practically inactive. If specific lytic antibody is produced by the rat, it is not present in the blood in sufficient quantities to be detected by this method. Furthermore, the simultaneous injection of serum from treated rats together with live trypanosomes into rats failed to modify the course of the disease. The duration of the infection in the rats receiving serum was the same as in the control rats.

2. *Effect of a Single Injection*

Gay and his associates (1923, 1926) have shown that an injection of certain substances, such as aleuronat (starch and gum arabic) broth, into the pleural cavity of rats produced an exudate in which the cells in 24 hours were predominantly polymorphonuclear, and in 48–72 hours were predominantly mononuclear. Animals so treated were protected against many times the fatal dose of a streptococcus culture injected into the pleural cavity 72 hours after the preparatory treatment. Nakahara (1925) obtained protection against pneumococci in mice and Tudoranu (1926) in rabbits by preparing the peritoneum. Stuppy, Cannon and Falk (1928) found that after vaccination with

pneumococci the local immunity in the lungs was associated with the accumulation of histiocytes.

It seemed, therefore, desirable to ascertain the resistance of rats prepared with a single injection of dead trypanosomes to an infection with trypanosomes. A single intraperitoneal injection of broth, serum or a suspension of dead trypanosomes was followed one or more days later by an infection with trypanosomes. Injection of saline, broth and peptone solutions, as well as supernatant serum from infected rats, had no appreciable effect on the course of infection. However, a single preparatory injection of dead trypanosomes decidedly modified the duration of the infection in comparison with controls prepared with saline alone. The results are summarized in Table II.

It will be noted that in each experiment the duration of infection in the prepared animal was longer by 40 per cent. or more than in control animals treated with saline. Furthermore, the effect apparently persisted for some time, because infection on the 5th and 10th days gave approximately the same results as 24 hours after preparation.

The increased resistance noted in these experiments is obviously not the result of a specific immunity but of a mobilization of phagocytic cells which suppress the number of trypanosomes and thus retard the progress of the infection. A study of the peritoneal exudate 24 hours after the injection of dead trypanosomes showed that there was an active mobilization of large mononuclears. A comparative study of the peritoneal exudate of the rat following the injection of saline, broth, serum and trypanosome suspensions, respectively, indicated that the last is the most powerful excitant. Saline had practically no effect. Broth and serum produced a marked increase in polynuclears and lymphocytes, but in no sense was the effect as striking as that produced by the trypanosome suspension. The abundance of large mononuclear cells in the peritoneal exudate 24 to 36 hours after the injection was so characteristic of the animals treated with trypanosome suspensions that they could be identified by the appearance of the exudate. This stimulation supports the view that the increase in resistance associated with the preparatory treatment was attributable to the hyperactivity of these cells.

3. *Effect of Splenectomy on the Course of Infection in Treated Rats*

The basis of the increased resistance was investigated from another angle. Rats given repeated injections of trypanosome suspension were splenectomized prior to infection, and the duration of illness was compared with non-splenectomized animals of the same group. A number of rats of the same weight were prepared in the same manner, and prior to infection half the number of animals were splenectomized, while the remaining half, as well as untreated normal and splenectomized animals, served as controls. Since the splenectomized animals had to be treated with neosalvarsan to prevent Bartonella infections, a number of normal animals were also treated with this drug. To ascertain the

response of rats of different ages to the immunization, two series of experiments were carried out with young rats, the treatment being started just at the end of the nursing period, that is, when the rats were about three weeks old.

TABLE II

Effect of a single injection of dead trypanosomes on the resistance of rats to infection

Experiment	No. of rats	Average weight	Interval between injection and infection	Incubation	Mean duration of infection	Material injected	Dose
1	6	59·8	½ hour	2·7	8·3	2 c.cm. supernatant serum	6,000
	5	59·6	½ hour	2·8	10·0	2 c.cm. trypanosome suspension	6,000
	4	58·5	½ hour	3·0	7·0	control ; saline	6,000
2	4		1 day	8·8	15·8	2 c.cm. trypanosome suspension	10,000
	3		1 day	3·7	8·4	control ; saline	10,000
3	4	66·4	1 day		10·4	2 c.cm. trypanosome suspension	12,000
	4	58·4	1 day		7·0	control ; saline	12,000
4	2		1 day	2·5	10·3	2 c.cm. trypanosome suspension	13,000
	2		1 day	2·0	7·5	control ; saline	13,000
5	3		1 day		9·7	trypanosome suspension	10,000
	3		5 days		10·0	trypanosome suspension	10,000
	2		control		6·3	control	10,000
6	7	86·4	1 day		14·4	trypanosome suspension	10,000
	7	73·0	10 days		15·6	trypanosome suspension	10,000
	8	78·8	control		9·9	control	10,000

The results are tabulated in Table III and analyzed in a note to that Table. These experiments bring out a number of interesting points. In the first place, they confirm our previous observations that old rats (60–100 gm.) are more resistant to infection than young ones (30–40 gm.), and that in the older rats splenectomy greatly reduced the duration of the infection, despite treatment with salvarsan. In the second place, they show that the immunization is as effective in young as in old rats, and that in both groups splenectomy results in a considerable decrease in resistance, the decrease being apparently more marked in the younger than in the older group of animals. Finally, in all experiments the immunized splenectomized rats still manifested a higher resistance than the splenectomized untreated animals, indicating that although the spleen is the principal organ of defence other organs also participate in the

defence against a trypanosome infection. These experiments strengthen the evidence that the immunity induced is due—largely at least—to a mobilization and activation of the reticulo-endothelial system.

It will be noted from the Table that a number of the immunized rats failed to develop an infection. This afforded an opportunity to study the duration of the immunity in these animals. In the first series of large rats, 3 (one received

TABLE III

Effect of splenectomy on rats immunized with trypanosome suspensions

SPLENECTOMIZED						NON-SPLENECTOMIZED			
No. of rats	Average weight	No. of injections	No. of rats	Incubation	Duration of infection; in days	No. of rats	Incubation	Duration of infection; in days	Remarks
9	135	10	4	8·5	14·9	5	7·0	17·0	2 infected—16·5 and 17·5 days; 3 remained neg.
10	124	control	5	3·0	6·4	2	5·0	12·8	Received salvarsan
						3	3·0	10·2	No salvarsan
8	53	10	4	8·7	11·7	2	7·0	17·0	1 remained neg., received salvarsan
						2	12·0	19·3	
10	37	control	4	2·8	6·5	3	3·7	7·2	Received salvarsan
						3	2·7	5·9	No salvarsan
16	95	10	8*	6·5	10·3	2	6·0	18·5	Received salvarsan 1 remained neg.
						6	6·6	18·5	2 remained neg.
16	94	control	8	3·5	7·2	3	6·0	13·7	Received salvarsan
						5	5·2	12·0	No salvarsan
19	36	10	9†	7·4	9·8	5	14·0	21·3	Received salvarsan 1 remained neg.
						5	5·2	13·1	No salvarsan
18	49	control	10	4·0	7·2	3	5·7	10·7	Received salvarsan
						5	4·6	9·7	No salvarsan

* = 2 remained negative. † = 1 remained negative.

NOTE TO TABLE III

		Duration of illness ; in days	Difference in duration of illness ; in days
Large rats :	Immune splenectomized ...	14·9	2·0
	„ non-splenectomized ...	17·0	
	Controls splenectomized ...	6·4	6·4
	„ non-splenectomized ...	12·8	
	Immune splenectomized ...	10·3	8·2
	„ non-splenectomized ...	18·5	
	Controls splenectomized	7·2	6·5
	„ non-splenectomized ...	13·7	
Small rats :	Immune splenectomized ...	11·2	5·8
	„ non-splenectomized ...	17·0	
	Controls splenectomized ...	6·5	0·7
	„ non-splenectomized ...	7·2	
	Immune splenectomized ...	9·8	12·1
	„ non-splenectomized ...	21·9	
	Controls splenectomized ...	7·2	3·5
	„ non-splenectomized ...	10·7	

salvarsan, the other two did not) failed to become infected. On July 17th, three weeks after the first infection, they were again infected with 15,000 trypanosomes. The control died in 8 days ; one of the immunized rats developed an infection and died after 13 days ; two remained negative. Among the small rats, one remained negative. This too was re-infected on July 17th ; the control died in 5·5 days, while the immunized animals failed to become infected.

On August 6th, these three rats were re-infected with 12,000 trypanosomes, with negative results.

On August 22nd, two of them were again infected, together with two controls. On August 24th, they were positive, one dying after 7 days, the other after 9·5 days. The controls died after 8 and 9·5 days respectively. These rats were, therefore, resistant to infection and re-infection for a period of two months after the last injection, but shortly thereafter lost their resistance completely. The transitory character of the immunity is of particular interest, and is now the subject of further study.

4. *Immunization of Guinea-pigs and Rabbits*

A series of guinea-pigs and rabbits were immunized in the same manner as the rats. The guinea-pigs received 10 injections intraperitoneally, while the rabbits were inoculated intravenously. After 10, and again after 20, injections the trypanolytic property of the sera was tested. The results are shown in Table IV.

It will be noted that of all the sera tested (rat, guinea-pig and rabbit) only those from the treated rabbits showed marked trypanolytic properties.

After 20 injections the immunized guinea-pigs were infected along with controls, but no differences were noted either in the incubation period or in the duration of the infection.

TABLE IV

Lytic properties of sera from animals immunized with dead trypanosome suspensions

Animals	Lytic effect			
	After 10 injections		After 20 injections	
	1 : 10	1 : 20	1 : 10	1 : 20
Guinea-pigs—				
III Immune	1,630	1,940	2,680	2,700
IV „	1,640	1,860	2,490	3,680
VII „	1,440	1,420	2,250	2,530
XI „	2,000	1,600	2,560	3,440
XII „	1,410	2,400	2,200	3,230
Control	2,500	2,300	1,800	3,230
„	1,800	2,100		
Rabbits—				
II Immune	20	90	390	1,810
III „	0	170	230	1,670
Control	2,300	2,400	3,230	3,130
Rats—				
1...	1,480	1,620		
2...	1,320	1,670		
3...	2,000	2,530		
4...	1,830	1,230		
Control saline	2,120	1,740		
„ „	1,740	2,100		
5 Immune			1,600	3,000
6 „			1,980	2,200
7 „			2,640	3,600
8 „			1,650	—
Control saline			2,100	2,780
„ „			2,900	2,700

5. *Passive Immunization of Rats*

Taliaferro and Johnson (1926) have shown that serum taken from infected guinea-pigs after a crisis exerted a therapeutic effect on mice infected with the mouse-passage strain. The results were irregular and zonal phenomena were observed, larger doses of sera often being less effective than smaller ones.

Table V

Effect of serum given at various intervals before and after infection

Experiment I

No. of rats	Amount of serum; in c.cm.	Time given	Infective dose	Duration of infection; in days
8	0·25 ser. 1	30 min. before	10,000	14·6
5	„ „ 2	„	„	16·2
1	„ „ 1	no count	„	13·5
1	0·25 ser. 1	12,000*	10,000	9·0
1	„ „	12,000	„	10·0
1	„ „	16,000	„	10·0
1	„ „	20,000	„	10·0
1	„ „	24,000	„	9·5
1	„ „	29,000	„	6·5
6				Average 10·0
6	Control			10·0
1	0·25 ser. 2	12,000	10,000	11·5
1	„ „	14,000	„	11·5
1	„ „	18,000	„	10·0
1	„ „	20,000	„	13·0
1	„ „	22,000	„	11·0
1	„ „	29,000	„	11·5
6				Average 11·4

Experiment II

No. of rats	Amount of serum; in c.cm.	Time given	Infective dose	Duration of infection; in days
5	0·25 mixed ser.	30 min. before	7,000	18·0
6	„ „	30 min. after	„	13·5
6	Controls		„	10·0

Experiment III

No. of rats	Amount of serum; in c.cm.	Time given	Infective dose	Duration of infection; in days
10	0·25 ser.	30 min. before	2,300	11·6 (12·0)
9	Control		„	7·5 (7·5)

*These numbers represent the number of trypanosomes (per c.mm.) in the blood of the treated rats at the time when the serum was given.

TABLE VI

Passive protection of normal and splenectomized rats with trypanolytic sera taken after a crisis

Experiment	No. of rats		Immune serum from guinea-pig; c.cm. injected		Average duration of infection; in days	Average weight in gm.
	Normal	Splenectomized	24 hours before	24 hours after		
I	6			0·25	13·5	
		5		0·25	12·2	
	6		0·25		19·9	
		6	0·25		16·8	
		5	Control		7·9	
		6	,, (with salvarsan)		9·5	
II		7	0·25		6·8	84–88 gm.
		7	Control		7·4	
	8		0·25		13·3	
	8		0·25		12·8	
	7 (with salvasan)		Control		10·7	
	6 (without salvarsan)		,,		11·7	
III	5		0·25 immune serum		9·2	53 gm.
	5			0·25 immune serum	11·2	
	5		normal serum		6·8	
	5			normal serum	7·1	
	5		normal serum		6·8	
		5	0·25 immune serum		8·3	
		5		0·25 immune serum	7·0	
		5	normal serum		6·4	
		5	no serum		6·6	
IV*	8		0·25 serum I		8·2	81–90 gm.
	8		,, ,, II		9·2	
	8		,, ,, III		11·4	
	8		Control		7·9	
		8	0·25 serum I		6·5	
		8	,, ,, II		7·0	
		8	,, ,, III		7·0	
		4	Control		6·5	
V*	5		0·25		12·1	74–80 gm.
	5		,, + salvarsan		11·6	
	5		Control + salvarsan		9·5	
	4		,, — ,,		8·9	
		5	0·25 + ,,		9·8	
		4	Control		7·8	
VI*			Immune serum from cats			30–40 gm.
	5		0·25		16·4	
	5		Control		13·2	
		4	0·25		9·8	
		5	Control		8·3	

*Serum given just before infection.

Consistent results were obtained only in the early stages of the infection when the number of organisms was small.

In repeating the above experiments with rats, we also noted that the results depended on the stage of the infection. Only in the early stages of the infection, when the number of organisms was small, was there a small transient reduction. In no case was sterilization achieved.

We, therefore, decided to study the preventive effect of such sera, or, in other words, their ability to induce a passive immunity to infection with a rat-passage strain sensitive *in vitro* to the lytic action of the serum. In all instances only such sera were used as possessed definite lytic properties in a dilution of 1 : 10 or 1 : 20.

The results are summarized in a series of Tables.

Table V presents the results of a series of experiments designed to determine the effect of sera given before and at various intervals after the infection. In the first experiments serum was given before the infection, during the incubation period, and after the trypanosomes invaded the peripheral circulation. It is apparent that lytic sera delay the onset of the infection if given shortly before or after the infection, but have no appreciable effect if given after the trypanosomes have invaded the peripheral circulation.

Table VI presents data extending the preceding experiments to include a comparison of splenectomized and non-splenectomized animals. The results support those of the preceding experiments with regard to non-splenectomized animals. In splenectomized rats, however, the effect is variable. If a relatively non-virulent strain is used (experiment I), together with a potent serum, marked protective effects are noted. In those experiments in which a more virulent strain was used the protective effect was much less marked, although the average duration of infection was always a little longer than in the corresponding controls. Incidentally, these experiments confirm our previous findings that splenectomy greatly lowers the resistance of rats to a trypanosome infection.

TABLE VII
Effect of large quantities of serum

Experiment	No. of rats	Amount of serum; in c.cm.	Time of injection	Infective dose	Duration of infection
I	2	2·0 immune serum	5 days before	14,000	16·3
	2	2·0 ,, ,,	2 hours after	14,000	16·0
	2	2·0 normal serum	5 days before	14,000	12·3
	2	2·0 ,, ,,	2 hours after	14,000	13·0
II	2	1·2 mixed serum	24 hours before	22,000	21·0
	2	1·2 ,, ,,	24 ,, after	22,000	18·3
	2	1·2 normal serum	24 ,, before	22,000	16·0
	2	1·2 ,, ,,	24 ,, after	22,000	14·3

Table VII presents the data of two experiments in which larger quantities of immune serum were used. The results do not differ essentially from those obtained when only 0·25 c.cm. serum was used.

6. *Specific Effect of Immune Serum*

It was naturally of interest to determine whether the protective effect noted in the preceding experiments was specific in character. Cross-protection experiments were, therefore, made with *T. gambiense*, the results of which are summarized in Table VIII.

TABLE VIII

Specific effect of trypanolytic sera

Immune serum	Infecting organisms					
	T. evansi			*T. gambiense*		
	No. of rats	Average weight	Duration of infection	No. of rats	Average weight	Duration of infection
T. evansi						
0·25 c.cm.	4	39·0	15·3	4	39·0	8·4
Control	4	40·0	12·9	4	39·0	8·9
T. gambiense						
0·25 c.cm.				4	41·0	14·5
,, ,,				4	31·0	12·2
Control				4	40·0	6·5
T. evansi						
0·25 c.cm.				4	43·0	7·6
T. evansi						
0·3 c.cm.	3	95·0	18·2	2	83·0	10·8
Control	3	92·6	16·2	2	92·0	13·2

It is clear that there is a specific retardation of infection, the sera affecting only the homologous species. In principle the specific effect of the trypanolytic sera is the same as in the case of bacterial anti-sera ; they are, however, incapable of preventing an infection.

DISCUSSION OF RESULTS

The discussion of humoral versus cellular defences in bacterial infections has had its counterpart in trypanosome infections. As in the former, so in the latter it now appears that both are involved, that each plays its part, but that it

is essentially a question of relative significance. It is now generally recognized that in bacterial infections the cellular elements play an important part (cf. Gay), and that a degree of active immunity may be brought about without demonstrable presence of humoral antibody (cf. Besredka, 1927; Bull and McKee, 1927; Neufeld, 1926). However, while in bacterial infections the relatively simple and stable antigenic character of the organism leads to a prompt appearance of specific antibodies, thus often obscuring the part played by the form elements in the defensive process, in trypanosome (parasitic ?) infections, though similar processes are apparently involved, due to the more complex and variable character of the organisms and to the slow antibody response, the rôle of the cellular or form elements is more dominant, and the significant part taken by them in the defence against the invading organisms is brought out more clearly.

The experiments reported above throw additional light on the immunity phenomena in trypanosome infections. In the case of rats, the results seem entirely consistent. One or more injections of a suspension of dead trypanosomes (vaccine) increases the resistance of the rats to an infection with the same organism. The resistance does not appear to be enhanced to a greater extent by 20 than by 10 injections. After repeated injections, the blood of 2 out of 10 rats tested contained demonstrable quantities of lytic antibodies. Four of the immunized rats actually resisted repeated re-infections, three of them for a period of two months.

It would appear from these experiments that in the rat the enhanced resistance is due chiefly to an activation of the reticulo-endothelial system. This conclusion is supported by the facts that (1) injection of dead trypanosome suspensions increased the resistance of the treated rats ; (2) injections of dead trypanosome suspensions mobilize the large mononuclear cells in the peritoneal cavity ; (3) finally, the loss of the enhanced resistance following splenectomy.

In this connection the findings of Cannon and Taliaferro (1931) in bird malaria offer a striking parallel. In bird malaria these authors have failed to demonstrate a lytic antibody, and they conclude that the defensive mechanism rests in the reticulo-endothelial system.

In the guinea-pig, the results are still doubtful ; thus far the repeated injections of suspensions of dead trypanosomes have in no way effected the course of the disease.

In the rabbit, the intravenous injections of the specific vaccine have resulted in the production of a specific lytic antibody, but the effect of this vaccination on the course of an infection requires further study.

With regard to passive immunity, two things seem clear. A specific lytic serum injected into rats prior to or shortly after the infection results in a retardation of the infection. This effect appears to be due to the direct lytic action of the serum ; at the same time it appears that here too the endothelial cells play a certain rôle, for the same serum is less effective in splenectomized than in normal animals. These results are in line with those reported by Rosenthal

and Spitzer (1924) on the curative action of human serum in blocked mice.

It may well be that the zonal effect noted by Taliaferro and Johnson (1926) was due to the relative state of exhaustion of the endothelial system. It is significant that this zonal effect was noted only when the infection was relatively heavy—in other words, when there was a greater variability in the degree of exhaustion of the reticular system in the different animals.

The evidence thus far accumulated points clearly to the conclusion that the major defence in trypanosome infections consists in the activation of the endothelial system. The short duration of the immunity in those rats which fully resisted repeated infection is also in harmony with this view. It is quite comparable with the relatively short duration of immunity in malarial infections.

CONCLUSIONS

Rats treated with 1, 10 or 20 injections of a suspension of dead trypanosomes manifest an increased resistance to infection with the rat-passage strain of this organism.

Four rats out of over 100 used in these experiments fully resisted an infection ; three of these remained immune for a period of two months, after which they reacted in the same manner as the control rats.

Repeated injections of dead trypanosome suspensions failed to elicit appreciable lytic body in rats and guinea-pigs. In rabbits the injections were followed by the appearance of active trypanolytic antibody.

Lytic sera, taken from infected guinea-pigs and cats after a crisis, produce a specific passive immunity in rats. This immunity was manifested by a prolongation of the duration of the infection ; at no time was infection prevented. In splenectomized animals the results were variable, but a slight degree of protection was noted, more marked when a strain of milder virulence was used.

The conclusion seems warranted that in the rat the enhanced immunity resulting from the injection of dead trypanosomes is due to an activation of the reticulo-endothelial system.

REFERENCES

ANDREWS, J., JOHNSON, C. M., and DORMAL, V. J. (1930). Lethal factors in experimental infections of *Trypanosoma equiperdum* in rats. *Amer. Jl. Hyg.*, **12**, 381.

BESREDKA, A. (1927). Local immunization. Baltimore.

BULL, C. G., and MCKEE, C. M. (1927). Respiratory immunity in rabbits. II : Intranasal infection and immunization with pneumococci. *Amer. Jl. Hyg.*, **7**, 627.

CANNON, P. R., and TALIAFERRO, W. H. (1931). Acquired immunity in avian malaria. III : Cellular reactions in infection and superinfection. *Jl. Prev. Med.*, **5**, 37.

CLARK, A. R. (1929). The rôle of clasmatocytes in protection against pneumococcus. *Arch. Path.*, **8**, 464.

DOERR, R., and BERGER, W. (1922). Beziehungen zwischen Virulenz und Vermehrungsgeschwindigkeit der Erreger. *Ztschr. f. Hyg. u. Infekt.*, **95**, 319.

GAY, F. P., CLARK, A. R., and LINTON, R. W. (1926). A histologic basis for local resistance and immunity to streptococcus. *Arch. Path.*, **1**, 857.

——— and MORRISON, L. F. (1923). Clasmatocytes and resistance to streptococcus infection. *Jl. Infect. Dis.*, **33**, 364.

KLIGLER, I. J. (1929). Susceptibility and resistance to trypanosome infection. VI : The course of the infection in splenectomized rats. *Ann. Trop. Med. & Parasitol.*, **23**, 315.

——— (1931). Susceptibility and resistance to trypanosome infection. VIII : *In vitro* demonstration of specific agglutinating and trypanolytic antibodies in the serum of infected guinea-pigs. *Ibid.*, **25**, 377.

——— and COMAROFF, R. (1929). Susceptibility and resistance to trypanosome infections. V : The resistance of rats to infection. *Ibid.*, **23**, 103.

——— and WEITZMAN, I. (1924). Experimental study of trypanosomiasis in Palestine. *Ibid.*, **18**, 437.

LINTON, R. W. (1929). The reticulo-endothelial system in protozoan infections. *Arch. Path.*, **8**, 488.

NAKAHARA, W. (1925). The function of macrophages in local resistance to bacterial infections. *Jl. Exper. Med.*, **42**, 201.

NEUFELD, F. (1924). Über die verschiedene Empfänglichkeit junger und erwachsener Individuen für Infektionen und ihre Ursachen. *Ztschr. f. Hyg. u. Infekt.*, **103**, 471.

——— (1926). Natural immunity in its significance for epidemiology. De Lamar Lectures, 1926-27.

ROSENTHAL, F., and SPITZER, F. (1924). Die Bedeutung des Retikuloendothels für den Mechanismus der trypanoziden Wirkung des Menschenserums. *Ztschr. f. Immunitätsforsch.*, **40**, 529.

SCHILLING, C., and SOLLAZZO, G. (1928). Wirken Trypanosomen, mit Immunserum behandelt, als Antigen ? *Ibid.*, **59**, 219.

STUPPY, G. W., CANNON, P. R., and FALK, I. S. (1928). Nature of local immunity in lungs of rabbits immunized against pneumococci. *Proc. Soc. Exper. Biol. & Med.*, **36**, 314.

TALIAFERRO, W. H. (1929). The immunology of parasitic infections. N.Y.

——— (1934). Some cellular bases for immune reactions in parasitic infections. *Jl. Parasitol.*, **20**, 149.

——— and JOHNSON, T. L. (1926). Zone phenomena in *in vivo* trypanolysis and the therapeutic value of trypanolytic sera. *Jl. Prev. Med.*, **1**, 85.

THOMSON, J. G. (1931). The question of immunity in man to protozoal diseases. *Proc. Roy. Soc. Med.*, **24**, 499.

TUDORANU, G. (1926). Le mécanisme de l'immunité contre la pneumocoque type III. *Ann. Inst. Pasteur*, **40**, 606.

THE INTERNAL ANATOMY OF THE BLACK-FLY, *SIMULIUM ORNATUM* MG.

BY

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CONTENTS

INTRODUCTION

A survey of the relevant literature reveals the fact that, except for a somewhat inadequate paper of Hungerford (1913), there is no detailed account of the internal anatomy of any single species of the family Simuliidae. That this deficiency should be made good is desirable in view of Blacklock's (1926) discovery of the rôle played by the African *Simulium damnosum* in the transmission of *Onchocerca volvulus* from one human host to another, and of the subsequent demonstration by Hoffmann (1930) of the transmission of *O. caecutiens* by *S. mooseri*. The transmission of *Leucocytozoon anatis*, a haemogregarine of domestic and wild ducks, by *S. venustum* reported by O'Roke (1934), which suggests that Simuliids may play a rôle in the transmission of protozoan blood parasites, is an additional reason for presenting the description of the internal organs of a black-fly.

S. ornatum does not normally attack man, but it has been recorded as attacking domestic animals by Steward (1932) and by Edwards (1920). However, comparison of its internal structure with that of *S. hirtipes*, *S. venustum*, *S. pictipes* and *S. monticola* reveals few differences, and the first two species are well-known ' blood-suckers ' in North America.

Specimens of *S. ornatum* were readily accessible to the writer, and it was felt that a description of the internal anatomy of this species would be serviceable and would be found to correspond with that of other members of the family.

Where the feeding habits of the Simuliidae are known, only the females are recorded as ' blood-suckers.' Little is known of the feeding habits of the males, except that (1) they do not suck blood ; (2) their mouth-parts are of the same general conformation as those of the female, but much weaker ; (3) they are occasionally found at rest on the inflorescences of shrubs, such as willows,

and in other similar situations. Arguing from these facts and from analogous cases in other blood-sucking Diptera, it is usually stated that the male Simuliids are ‘ nectar feeders.’ Internally the difference in the anatomy of the two sexes is slight, except as regards the reproductive systems. In view of the unimportance of the male as a possible vector, it is proposed in the present paper to describe more fully the internal organs of the female, and subsequently to note the main differences between those of the two sexes.

GENERAL

Females of *S. ornatum* are about 5 mm. in length when freshly emerged from the pupa and unfed. A few exceed this length by 1 mm., while starvation will produce individuals as small as 2·5 mm. Feeding and development of the gonads result in an increase in the size of the abdomen, the integument of which is weakly chitinized except for the tergites of the last three segments.

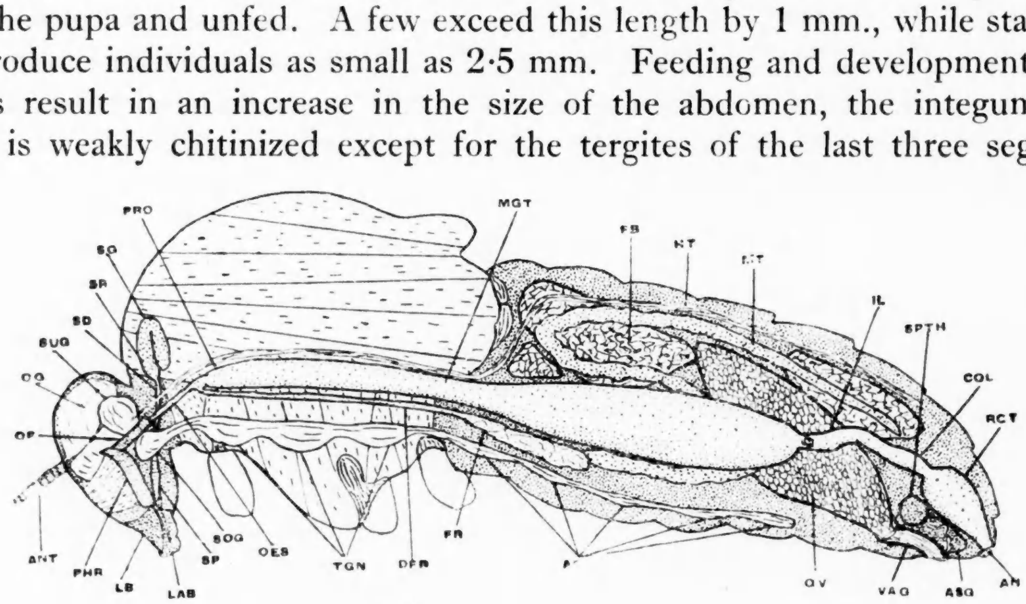


Fig. 1.

FIG. 1. Semi-diagrammatic section of unfed female *S. ornatum*, to show the relations of the internal organs. The ovary, Malpighian tubules, fat-body and salivary gland of the left side, and the parts of the brain of the left side, have been omitted. (× 20.)

Abbreviations (applicable to all figures). *AGN.*—abdominal ganglion ; *AN.*—anus ; *ANT.*—antenna ; *ASG.*—accessory gland ; *COL.*—colon ; *DFR.*—duct of food reservoir ; *FB.*—fat-body ; *FR.*—food reservoir ; *HT.*—heart ; *HYP.*—hypopharynx ; *IL.*—ileum ; *LAB.*—labium ; *LB.*—labrum ; *MGT.*—mid-gut ; *MT.*—Malpighian tubule ; *NC.*—nerve cord ; *OES.*—oesophagus ; *OG.*—optic ganglion ; *OP.*—oesophageal pump ; *OV.*—ovary ; *PHR.*—pharynx ; *PRO.*—proventriculus ; *RCT.*—rectum ; *RP.*—rectal papillae ; *SD.*—salivary duct ; *SG.*—salivary gland ; *SOG.*—sub-oesophageal ganglion ; *SP.*—salivary pump ; *SPTH.*—spermatheca ; *SR.*—salivary reservoir ; *SUG.*—supra-oesophageal ganglion ; *TGN.*—thoracic ganglion ; *VAG.*—vagina.

The general configuration of the fly can be seen in fig. 1, which is a somewhat diagrammatic sagittal section of an unfed female to show the relations of the various organs to each other and to the different regions of the body itself.

The mouth-parts are of the laterally-cutting type described by Jobling (1928) in *Culicoides*, and the oral aperture leads to a highly chitinized pharynx. The pharynx leads to the chitinized oesophageal pump, and thence the soft-walled oesophagus passes through the neck and expands slightly. From the floor of this expansion passes the duct of the ventral diverticulum, which itself lies in the antero-ventral part of the abdomen. Immediately posterior to the

expansion of the oesophagus is the oesophageal valve or proventriculus. The mid-gut extends from the proventriculus in the anterior part of the thorax to the fifth abdominal segment. It is a straight tubular structure divisible into a narrower anterior portion in the thorax and an expanded posterior portion in the abdomen, the latter being capable of considerable distension by food.

At the junction of the mid-gut and the hind-gut are four Malpighian tubules. The anterior two-thirds of the hind-gut are in the form of a narrow, slightly convoluted tube ; this passes into the expanded rectum, in which are six rectal papillae.

The ovaries are paired structures with a single spermatheca and a pair of accessory glands. The heart, nervous system and fat-body do not call for special mention. The salivary glands are situated in the anterior part of the thorax dorsal to the oesophagus.

THE MOUTH-PARTS

The mouth-parts of various species of Simuliidae have been figured and described by several authors, among whom may be mentioned Smith (1890), Emery (1913) and Cameron (1922). No attempt appears to have been made to determine the relationships of the various parts till Jobling (1928) showed that the mouth-parts of *S. ornatum* were arranged similarly to those of *Culicoides*. Examination in detail of the mouth-parts of *S. ornatum* and other Simuliid species has enabled me to corroborate Jobling's opinion, while observations made on living specimens of *S. hirtipes* demonstrated that the mode of biting was essentially the same as that of *Culicoides*.

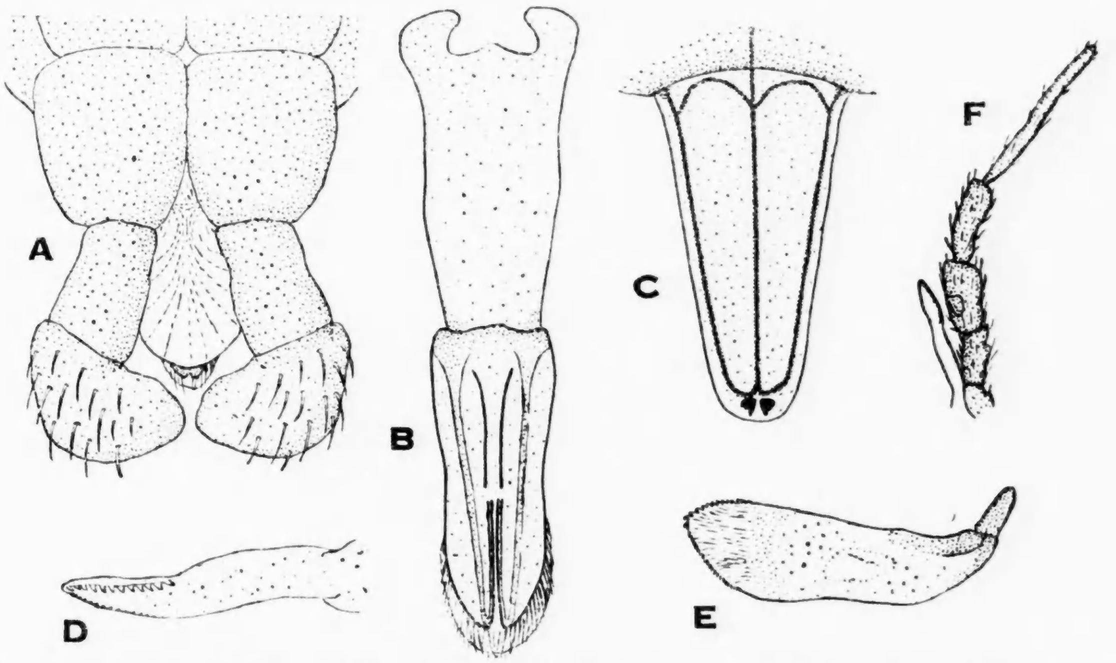


FIG. 2. The mouth-parts of the female of *S. ornatum*. *A.*—Labium, ventral view (× 130) ; *B.*—Hypopharynx, dorsal view (× 130) ; *C.*—Labrum epipharynx, dorsal view (× 130) ; *D.*—Right maxilla, dorsal view (× 130) ; *E.*—Right mandible, dorsal view (× 130) ; *F.*—Right maxilla and palp (× 57).

The various parts are shown in fig. 2. The labrum epipharynx (fig. 2, C) is a pointed, membranous structure, strengthened and kept rigid by three heavy, rod-like, surface chitinizations. At the tip are two heavily chitinized trifid teeth. The outer edges of the labrum are smooth.

The theca of the labium (fig. 2, A) appears to consist of two separate bilaterally symmetrical parts ; but actually these are fused at their bases, and only the labella are completely separate from each other. Proximally, the labella are stiffly chitinized, but distally they are soft and have numerous sensory hairs on their surfaces. They are capable of slight distension, and their extremities curve round and forward and almost meet in front of the labrum. When biting, the distal extremities of the labella surround the labrum and other mouthparts which are slowly inserted into the skin of the host, embraced and guided by the labella.

The hypopharynx (fig. 2, B) is a flat spatulate structure with a fringe of anteriorly directed bristles on its anterior margin. It is strengthened by various chitinizations shown in the figure ; the salivary duct opens about half-way down its length. It is not completely free from the labium, but is connected to it by a loose, sac-like, membranous structure lying between the proximal joints of the labella. Ventrally, within the sac-like structure, the hypopharynx has a keel-like ridge (fig. 4, *hyp.*), up which the salivary duct (*sd.*) passes.

The mandibles (fig. 2, E) are thin, flat structures. Their articulations are external to the outer margins of the labrum and the hypopharynx, but they are shaped in such a way as to have the greater part of their blades lying one on top of the other between the labrum and the hypopharynx. Their anterior margins are dentate and there are minute striations running up the blade from the marginal teeth. In the centre of the blade of each mandible is a clear depressed area similar to that found in *Culicoides* by Jobling (1928).

The maxillae (fig. 2, D) are somewhat lanceolate in shape when viewed from the dorsal (anterior) aspect. They are round in section, though this is modified distally where the anterior face is flattened, the retrorse teeth, with which it is armed, being set around this flattened part. The maxillae lie along the outer margins of the labrum and the hypopharynx. The palps (fig. 2, F) are four-segmented, though the first basal segment has the appearance of being the product of fusion of two, but it is quite rigid. The second segment bears a depressed flask-like sensory vesicle, which opens to the exterior.

THE SALIVARY GLANDS

The paired salivary glands (fig. 4, *sg.*) lie in the antero-dorsal region of the thorax (fig. 1, *sg.*). They consist of two parts, a distal, elongated, secretory portion consisting of large vacuolated cells, bent on itself in the form of a U, and a rounded reservoir (*sr.*) composed of smaller cells and from which passes the narrow salivary duct (*sd.*). The salivary ducts from the two glands pass down

on each side of the gut and the nerve cord, and unite beneath the latter. From the point of junction, the common salivary duct expands, and its walls are strengthened with rib-like chitinizations, the whole forming the salivary pump

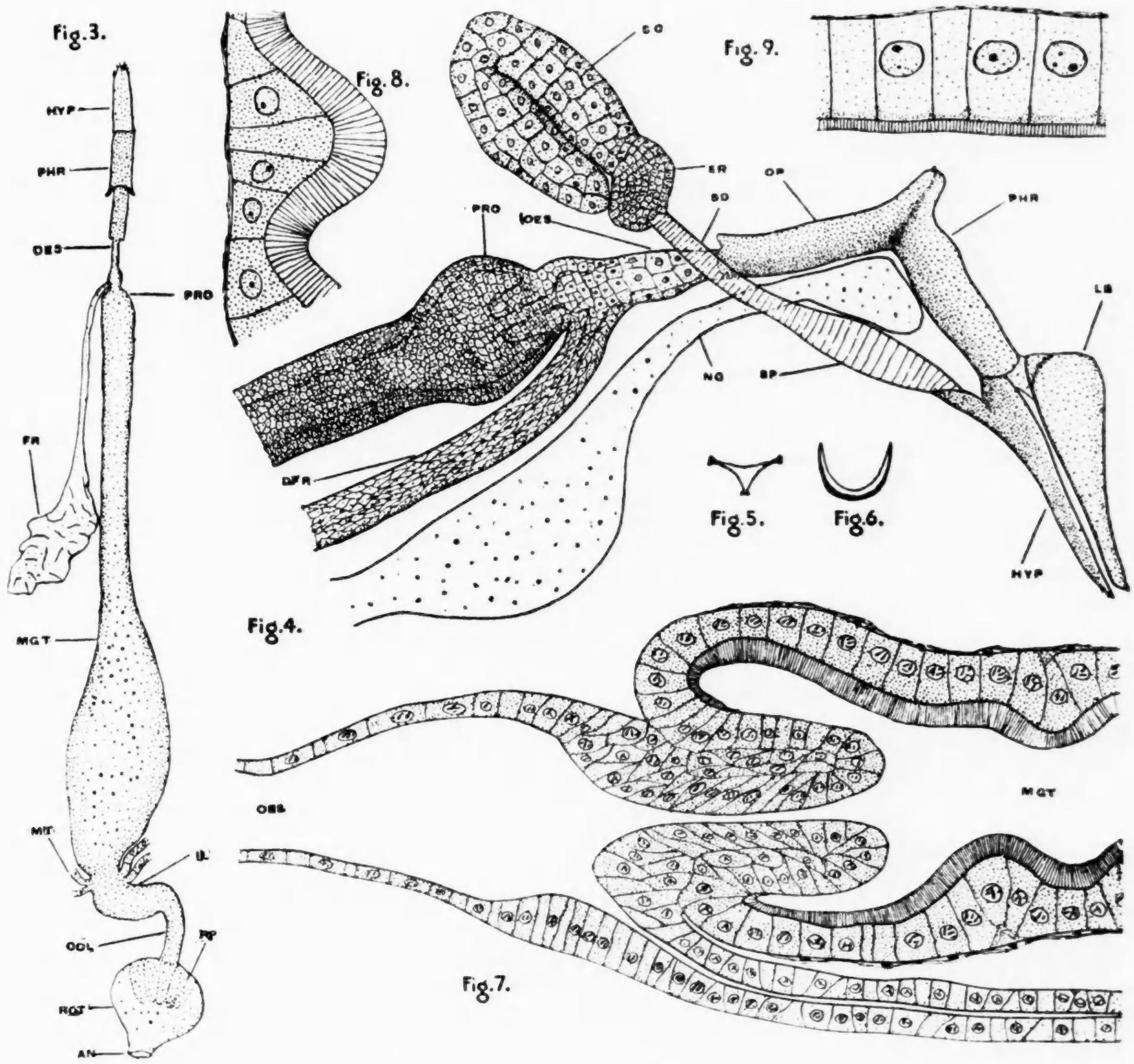


FIG. 3. The alimentary canal of an unfed female *S. ornatum*, dorsal view. (× 20.)
FIG. 4. The fore-gut, proventriculus and salivary glands, viewed laterally. (× 88.)
FIG. 5. Transverse section of the oesophageal pump. (× 88.)
FIG. 6. Transverse section of the pharynx. (× 88.)
FIG. 7. Longitudinal section of the proventriculus. (× 312.)
FIG. 8. Longitudinal section of the epithelium of the anterion region of the mid-gut. (× 600.)
FIG. 9. Longitudinal section of the epithelium of the posterior region of the mid-gut. (× 600.)

(*sp.*) which has been described by Nitzulescu (1927). Anteriorly, the common duct narrows and passes up the keel-like ridge on the ventral surface of the hypopharynx to open on the centre of its upper surface.

THE ALIMENTARY CANAL

From the mouth, which may be defined as the opening bounded by the bases of the epipharynx and the hypopharynx, the alimentary canal passes to the densely chitinized pharynx (*phr.*) and oesophagus. The pharynx is in the form of a broad tube with the dorsal wall invaginated, giving the whole a U-shape in traverse section (fig. 6). The ventral wall is densely chitinized, the dorsal one less so. Strong muscles are inserted in the dorsal wall. The anterior part of the oesophagus forms the oesophageal pump (*op.*) and is strongly chitinized, save at its immediate junction with the pharynx where it is membranous, thus permitting a certain flexibility of the joint. The oesophageal pump is triangular in section (fig. 5), and consists of two ventro-lateral chitinized plates and one dorsal chitinized plate. The pump is operated by muscles inserted on the plates, which are themselves elastic. The remainder of the oesophagus is thin-walled and passes into the thorax, where its walls thicken, and the lumen expands slightly before it joins the mid-gut in the oesophageal valve or proventriculus (fig. 4, *pro.*, and fig. 7).

On the floor of the oesophageal expansion mentioned above is the opening of the duct of the single median oesophageal diverticulum or food reservoir (*fr.*). The walls of the duct are very thin and collapsed, so as almost to obscure its lumen. The diverticulum itself (*fr.*) lies in the antero-ventral region of the abdomen below the mid-gut and above the nerve cord. In figures 1 and 3 the crop is shown in an empty and collapsed state ; it is, however, capable of considerable distension, and it has a fine irregular muscular meshwork on its walls.

The oesophageal valve or proventriculous (fig. 7) of the adult Simuliid is much simpler than that of the larva. Histologically, except for somewhat ill-defined differences in the shape and size of the component cells and the complete absence of a striated margin in the cells, the component tissues of the proventriculus are similar to those of the anterior portion of the mid-gut proper. No peritrophic membrane has been found in any of the species examined, and consequently in its absence the sole function of the valve would appear to be the prevention of the regurgitation of food. It does not act as an elaborate press for the production of the peritrophic membrane as in the larva, as remarked by Strickland (1913), Puri (1925) and Smart (1934).

The mid-gut extends from the proventriculus in the thorax to the fifth abdominal segment as a straight tube of varying diameter. The anterior half is narrow, while the posterior half is expanded to a degree that varies with the condition of the fly ; the expanded portion narrows posteriorly to join the hind-gut. The walls of the mid-gut have circular and longitudinal muscle-bands arranged in a fine but distinct rectangular meshwork. The epithelium of the anterior region of the mid-gut (fig. 8) is columnar, modified by being thrown into irregular folds. The depth of these folds is very variable among different specimens : in some it is hardly noticeable ; in others it is marked. The epithelium of the posterior region (fig. 9) is also variable in thickness ; it may

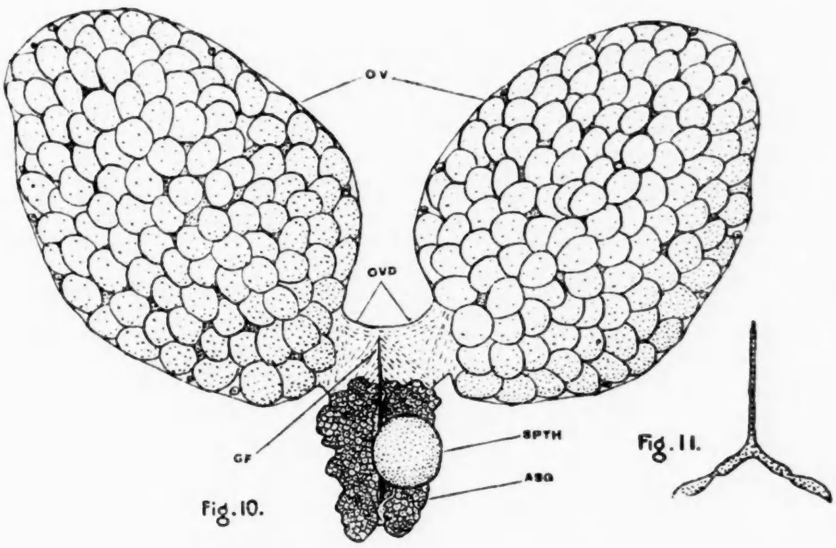
be as thick as that of the anterior region, or, when the gut is distended, it may be about a quarter of the thickness shown in fig. 9 ; it is usually without folds. The entire epithelium of the mid-gut has a striated margin, which is thicker in the anterior region.

The four Malpighian tubules enter the hind-gut at its junction with the mid-gut, in two lateral pairs, each pair having a common duct for entry. The tubules are approximately one-and-a-half times the length of the abdomen. The cells of the tubules are large with opaque granular contents and a large clear nucleus. The individual cells expand between the junctions with their neighbours, giving the tubule a coarse moniliform appearance, except at the basal end where the cells are smaller. A slight constriction of the gut occurs both above and below the openings of the Malpighian tubules.

The anterior two-thirds of the hind-gut (figs. 1 and 3, *il.* and *col.*) are tubular and slightly twisted. The twist, however, disappears later when the abdomen elongates as the result of the development of the ovaries. The posterior third of the hind-gut is expanded into a flask-shaped rectum (*rct.*) containing six rectal papillae, which project into the cavity and are arranged in a circle around the opening of the colon. The rectum narrows as the alimentary canal proceeds to the anus (*an.*), which is situated at the tip of the slightly recurved abdomen.

THE REPRODUCTIVE SYSTEM (FEMALE)

The ovaries (figs. 1 and 10) are of a similar type to those of *Chironomus* described by Miall and Hammond (1900). They consist of a large number of



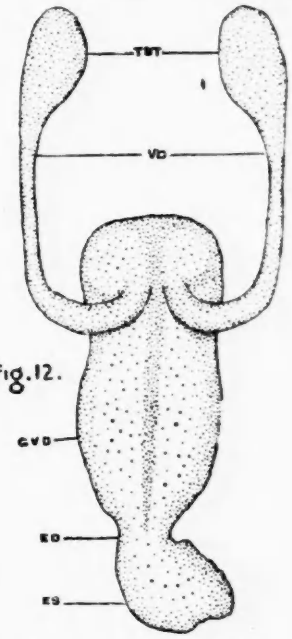


FIG. 10. The internal genitalia of the unfed female *S. ornatum*, dorsal view. (× 52.)
FIG. 11. Genital fork of female *S. ornatum*. (× 52.)
FIG. 12. The internal genitalia of the male *S. ornatum*. (× 52.)

ED.—ejaculatory duct ; *ES.*—ejaculatory sac ; *GF.*—genital fork ; *GVD.*—glandular part of the vas deferens ; *OVD.*—oviduct ; *TST.*—testis ; *VD.*—vas deferens.

short ovarioles radiating from a central axis, the whole surrounded by a thin membrane. The figures show the ovaries of recently emerged and unfed females. As they mature, the ovaries grow till they occupy practically the entire cavity of the abdomen, which becomes considerably distended with their growth. The oviducts at the bases of the ovaries are very short and membranous ; they lead to the vagina (*vag.*).

The vagina is flattened in the horizontal plane, its walls are muscular and there is a chitinous supporting rod (*gf.*) in the dorsal wall. This supporting rod is part of the ‘ genital fork ’ (fig. 11), which is an ingrowth of the cuticle at the external opening of the vagina, where it bifurcates into two arms which are continuous with the external cuticle. There is a single brown chitinous spermatheca (*spth.*), which lies dorsal to the vagina. Close to the spermatheca are a pair of accessory glands (*asg.*), whose ducts, along with that of the spermatheca, open to the exterior between the two arms of the genital fork.

Growth of the ovary, as noted above, leads to a considerable increase in size. The egg, when laid, is ·30 mm. by ·17 mm. by ·16 mm. and is triangulate in shape. In the fully developed ovaries of the ovipositing female most of the space is occupied by fully developed eggs. Microscopic examination, however, reveals the presence of immature ova, a finding which lends support to the conclusions of Pomeroy (1916) and Cameron (1922) that a second oviposition is probable.

The genital armature consists of two simple lateral flaps.

OTHER INTERNAL ORGANS

Other internal organs do not show any special peculiarities requiring mention. The heart lies in the position indicated in fig. 1 (*ht.*). The fat-body is scattered throughout the abdominal cavity ; it decreases in bulk as the ovaries develop. The central nervous system (*cns.*) consists of oesophageal ganglia, three thoracic ganglia and five abdominal ganglia.

THE ANATOMY OF THE MALE FLY

Except for the reproductive system, the various organs of the male fly differ but little from those of the female. The mouth-parts are slightly smaller and much weaker. The same parts are present as in the female, but the maxillae and mandibles are reduced to pointed stylets, armed with bristles instead of teeth, and they would appear to be useless as a cutting mechanism. The salivary glands are smaller than those of the female but otherwise similar. The gut exhibits no differences ; the Malpighian tubules are not as large as those of the female. Heart, fat-body and nervous system are as in the female.

The testes (fig. 12, *tst.*) are pyriform bodies lying dorso-laterally to the gut. They are invested with a brown fibrous sheath which is continuous with that of the vasa deferentia (*vd.*). The vasa pass round the gut and, ventral to it, become suddenly expanded and fuse together (*gvd.*). This fusion is external

only, and the lumina of the vasa remain distinct. The walls of the expanded portion of the vasa are glandular, of the type demonstrated in some other Diptera by Keuchenius (1913) ; they perform the functions of accessory glands which are absent. The expanded parts of the vasa are differentiated into two portions ; distally there is a rounded chamber into which the narrow parts of the vasa enter, the walls of which are less glandular than those of the proximal portions. The separate lumina unite to form a short ejaculatory duct (*ed.*) leading to an ejaculatory sac (*es.*), and thence to the external genital opening with its complicated copulatory armature.

Acknowledgments

The main part of the investigation was carried out in the Department of Zoology at the University of Edinburgh while the writer was holder of a Carnegie Research Scholarship. The writer's thanks are due to Professor J. H. Ashworth and Dr. A. E. Cameron, of Edinburgh, for their kind advice and help. Some preliminary observations and all those connected with the living flies were carried out while the writer was holder of a Research Scholarship from the Department of Agriculture for Scotland, studying at the New York State College of Agriculture at Cornell University, Ithaca, N.Y., U.S.A. ; the writer is indebted to Professor R. Matheson for his help at that time.

SUMMARY

The paper consists of a brief description of the internal organs of the female Simuliid as found in *S. ornatum* Mg. The general arrangement is very similar to that found in allied Diptera. The anatomy of the male fly resembles that of the female. Examination of specimens of four other species of *Simulium* shows that they resemble *S. ornatum* in the essentials of their internal organization.

REFERENCES

Baranov, N. (1927). Some morphological peculiarities of the family Simuliidae and their importance in the classification of this family. *Acta Soc. Ent. Serb.*, **2**, 19. (In Serbian, with a summary in German.)

Blacklock, D. B. (1926). The development of *Onchocerca volvulus* in *Simulium damnosum*. *Ann. Trop. Med. & Parasitol.*, **20**, 1.

Cameron, A. E. (1922). The morphology and biology of a Canadian cattle-infesting blackfly, *Simulium simile*, Mall. *Bull. Dept. Agric. Canada, new ser.*, no. 5.

Cragg, F. W. (1913). Studies on the mouth parts and sucking apparatus of the blood-sucking Diptera. II. *Sci. Mem. Off. Med. & San. Dept. Govt. India, new ser.*, no. 58.

Dimmock, G. (1881). The anatomy of the mouth-parts and of the sucking apparatus of some Diptera. pp. 1-50. Boston.

Edwards, F. W. (1920). On the British species of *Simulium*. II : The early stages. *Bull. Ent. Res.*, **11**, 211.

Emery, W. T. (1913). The morphology and biology of *Simulium vittatum*. *Kansas Univ. Sci. Bull.*, **8**, 323.

Grünberg, K. (1907). Die blutsaugenden Dipteren. pp. 114-17. Jena.

Hoffmann, C. C. (1930). Über *Onchocerca* im Süden von Mexiko und die Weiterentwicklung ihrer Mikrofilarien in *Eusimulium mooseri*. *Arch. f. Schiffs- u. Trop.-Hyg.*, **34** 461.

Hungerford, H. B. (1913). Anatomy of *Simulium vittatum*. *Kansas Univ. Sci. Bull.*, **8**, 365.

JOBLING, B. (1928). The structure of the head and mouth parts in *Culicoides pulicaris*, L. (Diptera Nematocera). *Bull. Ent. Res.*, **18**, 211.

KELLOGG, V. L. (1899). The mouthparts of the nematocerous Diptera. *Psyche, Camb., Mass.*, **8**, 327.

KEUCHENIUS, P. E. (1913). The structure of the internal gentalia of some male Diptera. *Ztsch. Wiss. Zool.*, **105**, 501.

MATHESON, R. (1932). Medical entomology. pp. 299-309. Springfield, Ill.

MEINERT, F. (1881). Fluernes Munddele, Trophi Dipterorum. pp. 41-43. Copenhagen.

MIALL, L. C., and HAMMOND, A. R. (1900). The harlequin fly.

NITZULESCU, V. (1926). Contribution à l'étude de l'appareil buccal des Simulides. *C. R. Soc. Biol.*, **95**, 1336.

——— (1927). Contribution à l'étude de la pompe salivaire des Simulides. *Bull. Soc. Path. Exot.*, **20**, 748.

——— (1928). Sur les tubérosités mandibulaires des Simulides. *C. R. Soc. Biol.*, **99**, 910.

O'ROKE, E. C. (1934). A malaria-like disease of ducks caused by *Leucocytozoon anatis* Wickware. *Bull. Sch. For., Michigan*, no. 4.

PATTON, W. S., and CRAGG, F. W. (1913). A textbook of medical entomology. Lond.

——— and EVANS, A. M. (1929). Insects, ticks, mites and venomous animals of medical and veterinary importance. Pt. I: Medical. L'pool.

PETERSON, A. (1916). The head-capsule and mouth-parts of Diptera. *Illinois Biol. Monogr.*, **3**, no. 2.

POMEROY, A. J. W. (1916). Notes on five North American buffalo gnats of the genus *Simulium*. *Bull. U.S. Dept. Agric.*, no. 329.

PURI, I. M. (1925). On the life-history and structure of the early stages of Simuliidae (Diptera, Nematocera). *Parasitol.*, **17**, 295.

SMART, J. (1934). On the biology of the black fly, *Simulium ornatum*, Mg. (Diptera, Simuliidae). *Proc. Roy. Phys. Soc.*, **22**, 217.

SMITH, J. B. (1890). A contribution toward the knowledge of the mouth parts of the Diptera. *Trans. Amer. Ent. Soc.*, **17**, 319.

STEWARD, J. S. (1932). A note on *Simulium* sp. attacking horses and cattle in Hertfordshire. *Rep. Inst. Anim. Path. Univ. Camb.*, **2**, 194.

STRICKLAND, E. H. (1913). Further observations on the parasites of *Simulium* larvae. *Jl. Morph.*, **24**, 43.

WILHELMI, J. (1920). Die Kriebelmückenplage. Jena.

INFECTION EXPERIMENTS WITH QUARTAN MALARIA

BY

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INTRODUCTION

It is a well-known fact that, up till now, only a few workers on malaria have succeeded in infecting *Anopheles* with *Plasmodium malariae*. Gordon and Davey in their critical review (1932) come to the conclusion that the experiment of Stephens and Christophers (1902) was the only successful one.

Judging by their criteria, I think that they should have discarded even the record of Stephens and Christophers, for, as they themselves point out, the examination of the patient's blood did not include the use of thick films, so that the possibility of a mixed infection must be borne in mind. Therefore, they might perhaps have replaced their third criterion, i.e., the exclusion of the possibility of a mixed infection in the host, by the requirement that the mosquitoes should be able to transmit quartan malaria to a healthy person.

I am convinced that the criteria of these authors are rather too severe and rigid, or at any rate that their application is so, as is their criticism of Hylkema (1920), whose claim they disallow because the oöcysts might not have progressed to sporozoite infection of the glands. Although Hylkema followed the oöcysts up to the stage of ripeness (with sporozoites visible within), the objection of Gordon and Davey might hold true but for the fact that Hylkema transmitted quartan malaria by his mosquitoes. It is true that this gland infection might have been at least partially the result of the natural infection ; but why, in the same mosquitoes, should only the natural and not the experimental infection progress to sporozoite infection ? At any rate, the experiments of Hylkema were sufficient to disprove the hypothesis of Marchoux (1930) that the quartan parasite is not transmitted by *Anopheles* mosquitoes, but that some other insect carrier is responsible for it.

Since Gordon and Davey's paper appeared, at least five records of indubitably positive results have been published (Anazawa, 1931 ; Mayne, 1932 ; Iyengar, 1933 ; Boyd and Stratman-Thomas, 1933 ; Mer, 1933). All these investigators used laboratory-bred mosquitoes and continued the experiments up to the sporozoite infection of the glands. Mer, as well as Boyd and Stratman-Thomas, even succeeded in infecting some patients with their mosquitoes.

MOSQUITO INFECTIONS

In connection with these positive records I should like to give a brief account of my own experiments with a quartan strain obtained from Vienna in September, 1933. (For the clinical account of the use of this strain in the

malaria therapy service at Amsterdam, see Winckel, 1934.) Two patients were inoculated subcutaneously with this strain on September 8th, and developed their first malarial attack on October 6th. The blood of one of these two already contained some gametocytes on October 9th, and on October 11th the number of male gametocytes was 6 per 500 leucocytes, counted in the thick film ; the number of females may have been approximately the same, but, as it seldom happens that the female gametes can safely be recognized in the thick film, I have decided as a routine method to count only the males. I hope to set forth in another paper my reasons for using the thick film in spite of this drawback. As the number of 6 per 500 is somewhat over the density of 1 per 100—which I have accepted as the minimum density for practical use—I then decided to apply a large number of mosquitoes to this patient. The mosquitoes used were wild *Anopheles maculipennis* var. *atroparvus* caught in a stable in the neighbourhood of Amsterdam, in a district which is at present free from malaria. Some 60 mosquitoes became engorged and were separated as batch no. 163 and kept in the tropical chamber of our Institute (temperature 27° C., relative humidity 90 per cent.). On October 14th, they were given the opportunity of a second blood-meal on the same patient (3 ♂ per 1,000 leucocytes). Most of the mosquitoes satiated themselves, whereafter the whole batch was replaced in the tropical chamber. After this they were fed only on sugar-water. On October 17th, two were killed and one of these had 4 very small oöcysts. From October 19th till October 24th, six mosquitoes were dissected, two of which had positive stomachs (1 and 4 oöcysts). On October 26th, two mosquitoes were examined and showed infection, both of the stomach (1 and 1 ripe cyst) and of the glands. On the following day, out of five mosquitoes none showed stomach infection and only one had sporozoites in the glands. The results of the dissection of the remaining mosquitoes may be seen in the Table.

Since this first, unexpectedly successful, experiment I have tried more than once to repeat it, but with poor results, as is shown by the Table. In one batch, only five mosquitoes were found to harbour sporozoites in their glands ; in four other batches, a few positive stomachs were found. All these stomach infections were very light, except in batch no. 172, where one stomach showed as many as 150 oöcysts. This is one of those remarkable cases which we find occasionally in experimental malaria infections. The only reasonable explanation seems to be that the number of gametocytes in the patient's blood was sufficient, but that the bad results in the other mosquitoes are caused by something in the physiology of their stomachs.

As there is the possibility of *A. maculipennis* not being a favourable host for the quartan parasite, I also made some experiments with *A. bifurcatus*, of which I had recently discovered a profuse breeding-place in the neighbourhood of Amsterdam. However, the mosquitoes bred from pupae caught there gave the same bad results ; and it was proved by these experiments that *A. bifurcatus*, at least in the autumn, are too short-lived to be of any practical use in this work.

TABLE

Results of infection experiments

Serial no. of batch	Date of infecting feed	Number of ♂ gametoc. per 1,000 leucoc.	Stomachs		Glands		Numbers of oöcysts	Remarks on *Anopheles*
			dissected	positive	dissected	positive		
163	Oct. 11 & 14	12–3	8	3	45	12	4–1–4	wild *atroparvus*
169	Jan. 23 & 25	9–10	24	0	—	—	—	,, ,,
170	,, 26	—	14	0	—	—	—	,, ,,
172	Feb. 26	6	33	3	21	0	10–15–150	,, ,,
174	Mar. 19 & 21	9–8	9	2	35	5	1–3	,, ,,
178	May 29	3	18	0	—	—	—	lab.-bred *atroparvus*
181	June 8	9	19	0	—	—	—	,, ,,
184	Sept. 11	3	5	0	—	—	—	*bifurcatus*
185	,, 11	3	37	0	—	—	—	lab.-bred *atroparvus*
187	,, 25 & 28	7–10	16	2	—	—	1–2	*bifurcatus*
188	,, 25 & 28	7–10	11	1	13	0	1	lab.-bred *atroparvus*
189	,, 28	10	22	0	—	—	—	,, ,,
193	Feb. 9, 11 & 13	5–9–3	37	4	9	0	1–6–1–2	wild *atroparvus*
194	,, 13	3	31	0	—	—	—	,, ,,
		Total	284	15	123	17		

Remarks

1. The batches which are bracketed together were fed on the same gametocyte carrier.
2. The total number of dissected mosquitoes in each batch is found by adding the figures for stomachs and glands. From the 15th day onwards the glands, as well as the stomachs, were dissected, but the stomachs are not included in the numbers of the Table.

HUMAN INFECTIONS

Batch no. 163 was used for the inoculation of two volunteers and two patients with general paralysis. These four cases may be summarized as follows :

Case 1. Volunteer S., bitten by one fairly heavily infected mosquito on October 27th. On November 21st a rise in temperature to 38·9° C. occurred. Next morning the thick film revealed quartan parasites (12 per 500 leuc.). Quinine therapy was commenced on November 23rd. Parasites were still observed on the two following days, but on November 26th the blood was negative.

Case 2. Volunteer W., bitten by one moderately infected mosquito on October 31st. On November 24th the first quartan parasites were detected in the thick film (2 per 1,200 leuc.). On the evening of November 28th he complained of a feeling of shivering, but there was only a very slight rise of temperature to 37·4° C. On December 1st the density of the parasites had risen to the ratio of 2 per 100 leuc. and the temperature rose to 39° C. Atebrin therapy was then commenced (on the first day, one tablet ; on the following 4 days, 3 tablets ; and on December 6th, 2 tablets). On December 4th there was a second definite paroxysm (39·8°), the parasites having become even more numerous (8 per 100 leuc.). On December 8th the blood had become negative.

Case 3. G.P. patient, bitten by most of the mosquitoes of the batch, of which some 6 or 7 may have been positive. On November 21st the first paroxysm occurred (40·3°) and quartan parasites were found (2 per 1,000 leuc.). After two more paroxysms at quartan intervals the fever became of duplicate type.

Case 4. G.P. patient, bitten by 32 mosquitoes on November 23rd. On dissection 7 of these proved to have sporozoites in the glands. On December 16th, quartan parasites were found. On December 18th the temperature rose to 39·4°, and thereafter the patient had 10 paroxysms at quartan intervals.

DISCUSSION

It follows from the mosquito-infection experiments that the 'extrinsic' incubation period is 15 days, because :—

1. The presence of some ripe oöcysts in the first batch on October 26th indicates that the gland infection cannot be much older than 12 hours, for in such light infections as this there is always a very marked simultaneity in the ripening of the oöcysts.

2. The assumption that these gland infections are the result of the second blood-meal is not justified, for in that case the incubation period would be only two days longer than in benign tertian, where it has a duration of 10 days under the same conditions ; this would seem to be contradicted strongly by the results of other authors.*

The first very small zygotes could be detected after 6 days, whereas in benign tertian this happens after 3–4 days.

The ' intrinsic ' incubation period has a duration of 24–25 days ; in case 2 it was 28 days (or 31 days, if the first slight rise in temperature is to be neglected). It is noteworthy, however, that even in this case the first parasites were detected after 24 days.

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*Iyengar (1933, p. 852, serial no. 3 in the Table) records an incubation period of 10 days, but the author told me that this must be a mistake, since the very shortest incubation period which he found was 14 days.

If only the positive batches of the Table are taken into account, the total number of dissected stomachs is 114, positive 15. The total number of the glands is 123, positive 17. It is often suggested in accounts of unsuccessful experiments, where only a few zygotes were found in the first days, that this is due not to chance, but to the zygotes ceasing their development and disappearing. It is obvious that these figures do not support this view. In this respect it is interesting to notice that 2 of the 4 positive stomachs of group 193 were dissected on the 15th and 16th day after the second infecting meal and yielded some ripe cysts filled with sporozoites. Moreover, I have always found (in work on benign tertian) that young or old zygotes which cease their development do not disappear, but remain visible and easily recognizable as more or less degenerated cysts for weeks or months afterwards.

CONCLUSIONS

From these experiments I think that the following conclusions may be drawn :

1. The Vienna strain of malaria quartana develops only in rare cases a sufficient number of gametocytes to infect mosquitoes successfully.

2. Whereas in the present author's benign tertian strains the female gametocytes are mostly more numerous than the males, in this quartan strain this ratio seems to be a reverse one, so that, in using the number of males only as a criterion, we may expect negative results with gametocyte counts, which in tertian are a guarantee of success.

3. The negative results are not to be ascribed to some mystery in the life-history of *Plasmodium malariae.*

These conclusions, coupled with the fact that this is the first record of the experimental transmission of *Pl. malariae* by *Anopheles maculipennis* var. *atroparvus*, appear to the author to be of sufficient importance to justify this report.

REFERENCES

ANAZAWA, K. (1931). Experimental studies on the infectability of anopheline mosquitoes of Formosa. *Jl. Med. Assoc. Formosa*, **30**, 609.

BOYD, M. F., and STRATMAN-THOMAS, W. K. (1933). A note on the transmission of quartan malaria by *Anopheles quadrimaculatus*. *Amer. Jl. Trop. Med.*, **13**, 265.

GORDON, R. M., and DAVEY, T. H. (1932). *P. malariae* in Freetown, Sierra Leone. *Ann. Trop. Med. & Parasitol.*, **26**, 65.

HYLKEMA, B. (1920). The development of the parasites of quartan malaria in the *Myzomyia ludlowi* and their transmission on man. *Meded. Burg. Geneesk. Dienst Ned.-Ind.*, **6**, 50.

IYENGAR, M. O. T. (1933). Experimental infections of anopheline mosquitoes. *Ind. Jl. Med. Res.*, **20**, 841.

MARCHOUX, E. (1930). La fièvre quarte et son mystère. *Rev. Colon. Méd. Chir.*, Oct. 15th.

MAYNE, B. (1932). Note on experimental infection of *Anopheles punctipennis* with quartan malaria. *Publ. Health Rep., Washington*, **47**, 1771.

MER, G. (1933). Observations on the development of *Plasmodium malariae* Lav. in *Anopheles elutus* Edw. *Ann. Trop. Med. & Parasitol.*, **27**, 483.

STEPHENS, J. W. W., and CHRISTOPHERS, S. R. (1902). The relation of species of anopheles to malarial endemicity. *Rep. Malaria Comm. Roy. Soc.*, VII, 20.

WINCKEL, C. W. F. (1934). Kunstmatig opgewekte malaria quartana. *Ned. Tijdschr. Geneesk.*, **78**, 445.

SIMULIUM GRISEICOLLE BECKER, FROM THE SUDAN

BY

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CONTENTS

INTRODUCTION

Simulium griseicolle is a pest of considerable importance in parts of the Anglo-Egyptian Sudan in the region of the River Nile, where at certain seasons it appears in vast swarms and attacks both man and beast. The fly was originally described by Becker in 1903 from specimens taken at Assuan in Egypt, but its habits were first reported on from the Sudan in 1905 by the late Sir Andrew Balfour, and later in 1908 by Mr. H. H. King, who also investigated its breeding places. The communications of both authors are recorded in detail by Major Austen (1909).

The report of Sir Andrew Balfour is taken from a letter received from the Mudir of Berber, who states that human beings are chiefly bitten on the face. Mr. H. H. King's report is made from personal observations. He states that, so far as man is concerned, the insect's power to suck blood is very limited, and it appears to be unable to pierce the skin, except in such places as immediately behind the ear ; even in this situation it was never seen engorged with blood. It causes intense annoyance both by its habit of crawling into the eyes, ears and nose, and by continually pricking the skin in its ineffectual efforts to obtain blood. On a donkey, however, King observed the insect to feed greedily and to fly away with its abdomen enormously distended.

Morphologically *Simulium griseicolle* is of great interest. It has certain peculiarities of the larva, pupa and adult which are not found in any other Simuliid of the Ethiopian region. The unique character of the respiratory filaments of the pupa, which are leaf-like in shape, make it one of the most easily recognized ; the strikingly peculiar proleg of the larva with its terminal hooks situated on a lateral projection distinguishes it immediately, while the absence of hairs from the base of the radius of the wing (as noted by De Meillon in 1930) renders the identification of the adult a simple matter. Nevertheless, the fly may be one of a small group of closely related species—as the writer has shown to be the case with many Simuliids in Uganda—and the subsequent

confirmation of its identification by the use of secondary characters is a procedure to be aimed at.

The present contribution to the study of Ethiopian Simuliidae is based on material kindly placed at the writer's disposal by Mr. H. H. King, Government Entomologist of the Anglo-Egyptian Sudan. Fortunately, amongst the collection were mature larvae and pupae whose identification were confirmed by dissection. It is therefore possible to deal with the early stages as well as with the adults of both sexes in this paper.

FEMALE

(Described from three specimens taken by Mr. H. H. King. Two are labelled Khandak, 28.1.12, and the other was taken in Khartoum from the office window, 10.4.12.)

A light-coloured species.

Length 2 mm. ; wing 2·2 mm.

Head. Vertex, front and clypeus slate-grey, covered with narrow pale yellow scales ; front of normal width. Antenna eleven-segmented, dark brown with the exception of the scape and basal flagellar segment, which are ochraceous and covered with a fine ash-grey pubescence.

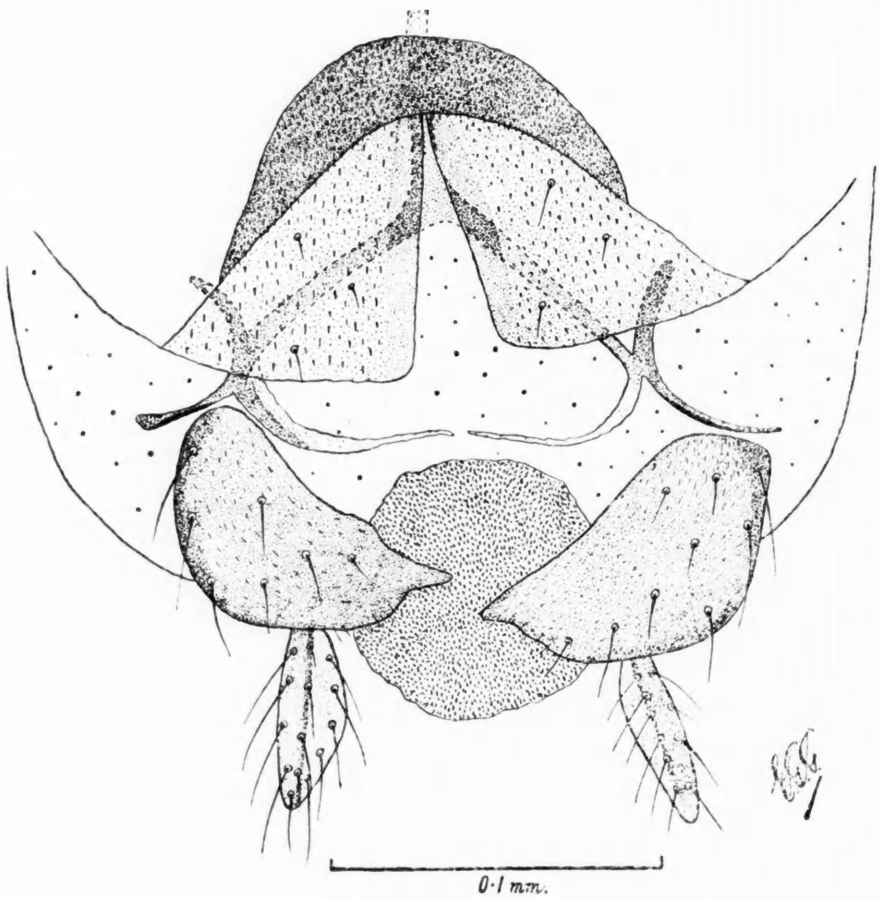

FIG. 1. Female terminalia, ventral aspect.

Thorax. Integument of mesonotum slate-grey, with closely applied silvery-grey scales. Scutellum with long yellow marginal hairs and pale yellow scales. Pleura slate-grey, without hairs on the membrane behind the thoracic spiracle. Halteres deep yellow, with base of stem brown.

Abdomen. Dark brown, densely covered with long silvery-grey scales ; basal fringe with long pale hairs.

Terminalia (fig. 1). Eighth sternite with a large median chitinized area which is not raised in the form of a plate ; anterior gonopophyses forming two right-angled triangles, lightly chitinized and with a few stout setae ; paraprocts with an inner projection. The cerci are long and narrow in ventral aspect.

Legs. Yellowish-brown, with the exception of the tarsi and apex of the tibiae which are brown ; calcipala present and pedisulcus distinct ; claws with a pronounced basal projection.

Wings. Pale yellow anteriorly, without basal cell ; costa with hairs interspersed with spines ; subcosta, base of radius and Rs without setae. Rs simple ; Cu_2 with a distinct double bend.

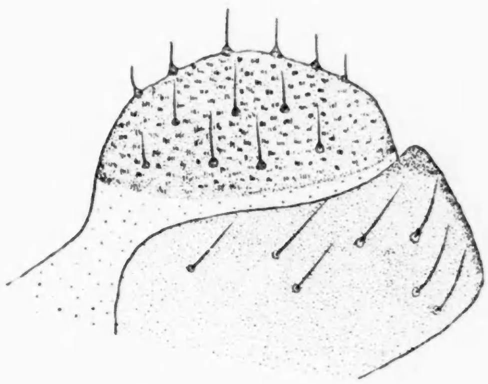

FIG. 2. Female terminalia ; lateral view of paraproct and cercus. (Magnification as in fig. 1.)

MALE

(Described from three specimens taken by Mr. H. H. King. All were collected from Argo Island, two on January 18th, 1915, and the other on February 4th, 1911.)

Length 2·1 mm. ; wing 2·2 mm.

Head. Eyes closely approximated. Front and clypeus light grey, the latter with sparse short grey hairs. Antenna as in the female, with the exception of the scape and basal flagellar segment which are light brown.

Thorax. Integument of mesonotum slate-grey, with a broad velvet-black median area uniformily and densely covered with closely applied long narrow golden scales; those in the median area deeper in colour. Scutellum velvet-black, sparsely covered with long narrow golden scales and with sparse marginal hairs. Pleura and halteres as in female.

Abdomen. Dark brown, with sparse golden scales ; basal fringe with long pale hairs.

Terminalia (fig. 3). Coxites slightly longer than the styles, narrow and cylindrical with long stout setae. Styles long and tapering in the anterior third to a stout strongly chitinized tooth-like projection. Intercoxal piece as in fig. 4, *a*, with two basal strongly chitinized pointed processes. Phallosome (fig. 4, *b*) with a pair of remarkable parameres attached on either side, each with ten horn-shaped processes of varying length projecting anteriorly. Cerci and tenth segment as in fig. 5, *a*.

Legs. Brown ; tarsi a darker brown than the other segments.

Wings. As in the female.

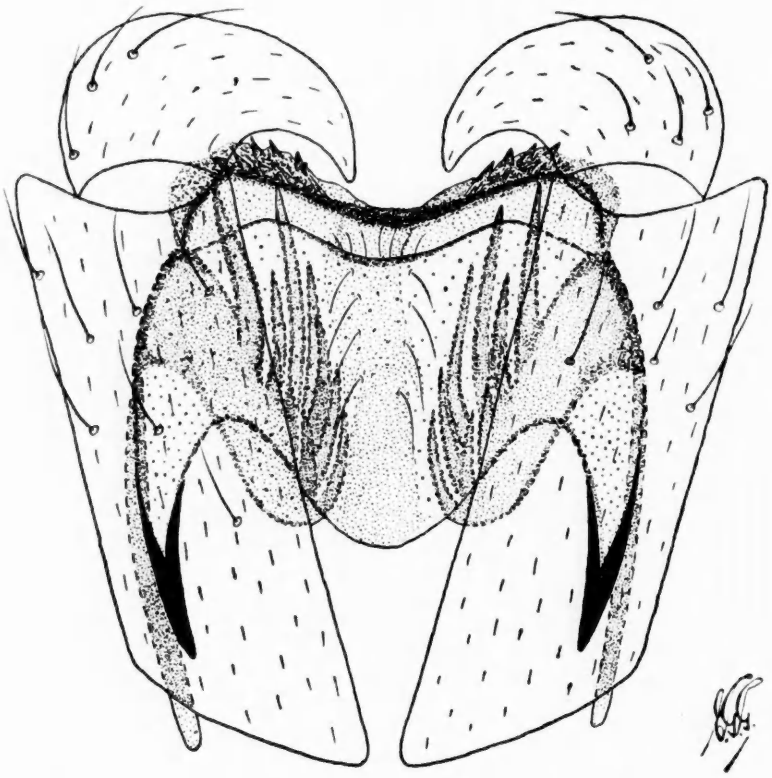

FIG. 3. Male terminalia, ventral view ; note that the tooth-like projection of the style is not seen when the terminalia are examined uncompressed from a ventral aspect. (Magnification as in fig. 1.)

LARVA

(The early stages are described from specimens collected by Mr. H. H. King at Abu Fatma on January 10th, 1912.)

Length of mature larva 4 mm. General colour ashy-grey to greenish. Body covered with short spines.

Head. No pigmented areas visible. Antenna normal, slightly longer than the base of the feeding brushes. Mandible (fig. 6, *a*) with a main tooth surmounted by two smaller teeth and with five small teeth protruding from its

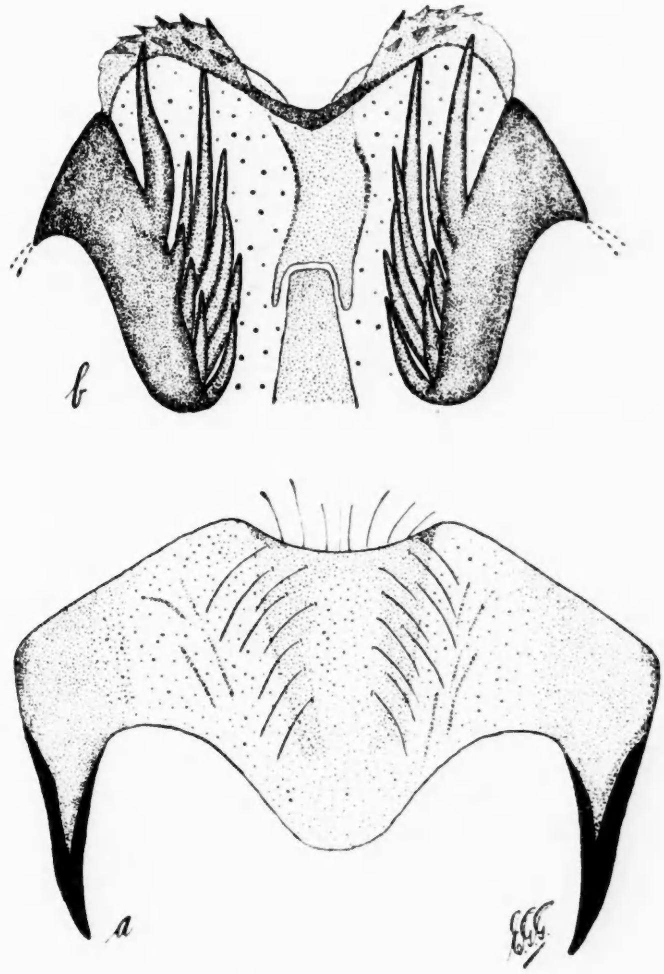

FIG. 4. Male terminalia. *a.*—Intercoxal piece ; *b.*—Phallosome and parameres ; dissected out. (Magnification as in fig. 1.)

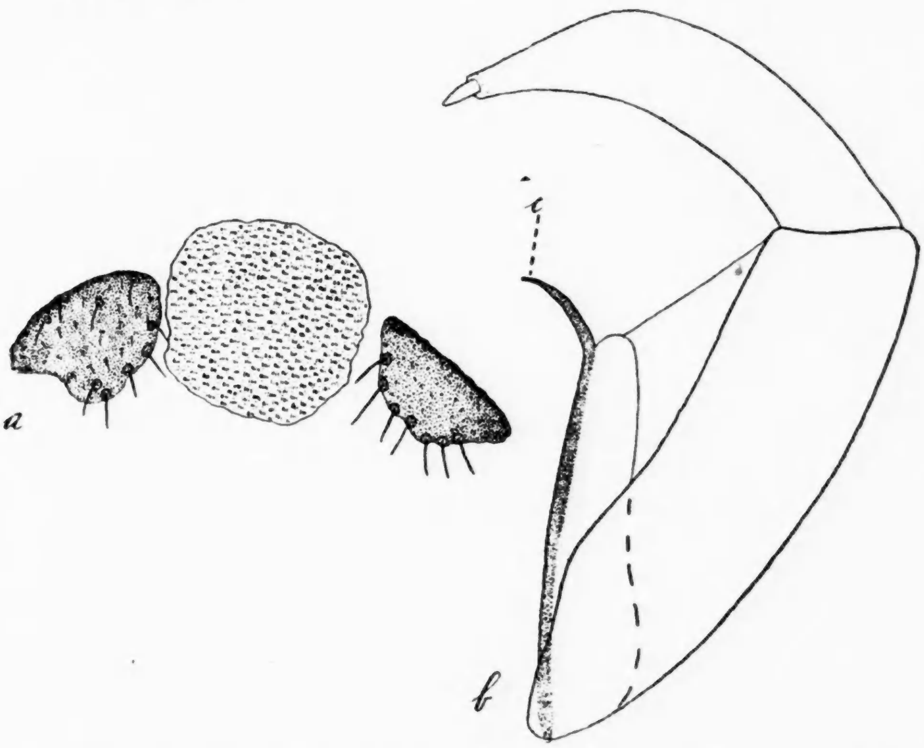

FIG. 5. Male terminalia. *a.*—Cerci and tenth segment ; *b.*—Coxite and style in lateral view ; *c.*—attachment of phallosome. (Magnification as in fig. 1.)

concave surface. Mentum (fig. 6, *b*) with a terminal row of thirteen heavily chitinized teeth, the median teeth shorter than the rest and the outer pair situated laterally ; all except the outermost, which are sharp and short, are roundly pointed. Feeding brushes with about 30 long bristles.

Thorax. Pseudopod (fig. 6, *c*) of peculiar shape. The usual terminal sucker is situated on a lateral projection ; it carries comparatively few hooks and is armed with long stout spines at the tip.

Abdomen. Anal gills trilobed, the median lobe with five and the lateral with eight finger-like processes. Anal armature normal. Anal sucker with about eighty rows of 12–15 hooks.

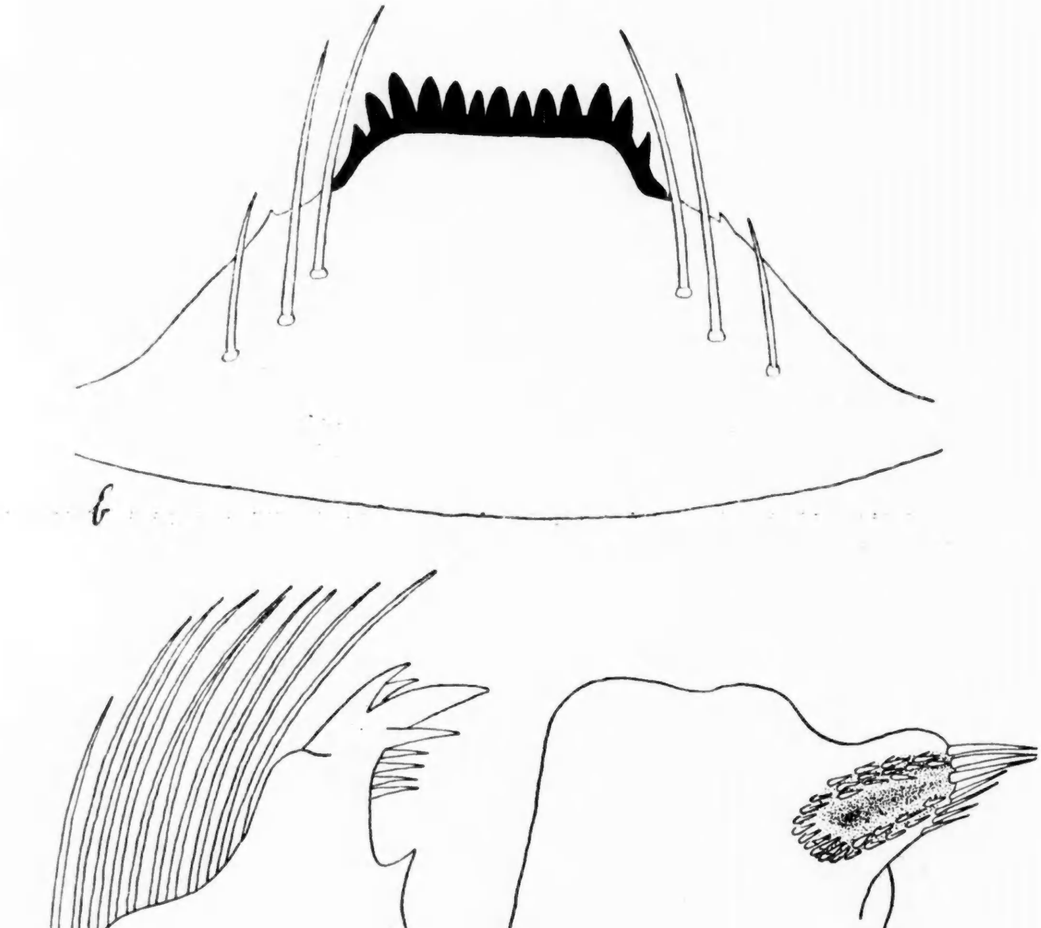

FIG. 6. Larva. *a.*—Mandible ; *b.*—Mentum ; *c.*—Anterior end of pseudopod. (Magnification as in fig. 1.)

PUPA AND COCOON

Head and *Thorax.* With disc-like tubercles ; trichomes (fig. 7, *a*) branched. In some cases all six branches arise from a short broad main stem ; in other

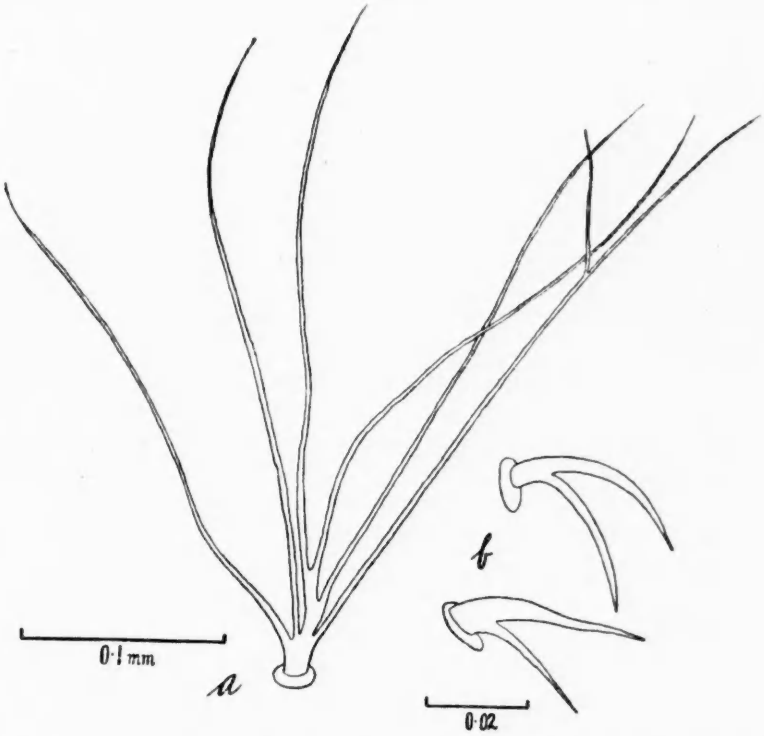

FIG. 7. Pupa. *a.*—Trichome ; *b.*—Bifid hooks of second abdominal segment.

cases the filaments arise as figured. The respiratory organ (fig. 8) consists of three long, narrow, scooped, semi-transparent, leaf-like processes, two of which arise from a single broad base, about half as long as the cocoon.

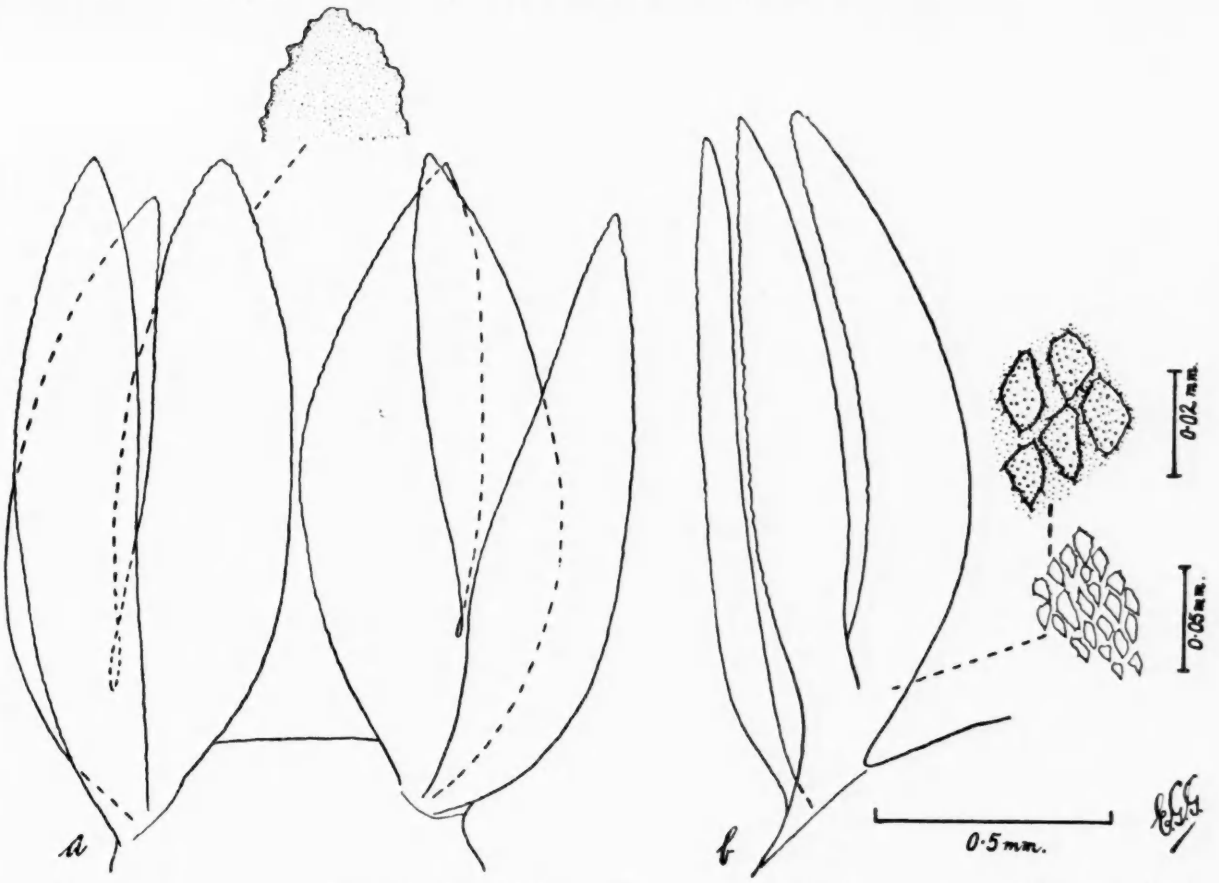

FIG. 8. Pupa. *a.*—Ventral view of the respiratory organ in its natural position attached to the pupa ; *b.*—Lateral view showing the scooped appearance of the organ.

Abdomen. With a pair of short, downwardly projecting terminal hooks. Dorso-lateral surface : first and second segments with four equally spaced simple hooks. Ventro-lateral surface : second segment with a pair of bifid hooks (fig. 7, *b*) ; fourth and fifth with a simple outer hook and a bifid inner hook ; sixth and seventh segments with a single simple hook.

The cocoon is an untidy gelatinous structure interwoven with coarse threads 3 mm. long.

REFERENCES

AUSTEN, E. E. (1909). Illustrations of African blood-sucking flies. p. 31. Lond.

DE MEILLON, B. (1930). On the Ethiopian Simuliidae. *Bull. Ent. Res.*, **21**, 185.

A NEW SPECIES OF AVITELLININE TAPE-WORM, *AVITELLINA SANDGROUNDI*, FROM *HIPPOTRAGUS EQUINUS*

BY

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(*Received for publication* 4 *April*, 1935)

Avitellina centripunctata, the type species, was first recorded from Italian sheep, with a very brief description of external features, by Rivolta in 1874 (under the name of *Taenia centripunctata*) and was first described in some detail by the present writer in 1927. Since Rivolta's original description, this species has been reported to occur in sheep* in South Africa by Gough (1911), Veglia (1919), Baer (1926, 1927) and Mönnig (1928), in Algeria by Neumann (1891) and in India by Pease (1903) and Bhalerao (1934). It is to be remarked, however, that all these reports, except Gough's, appear to have been based solely on superficial examinations of the more or less entire worms and not on study of sectioned material, and, if so, we are devoid of any proof that these Avitellinine species were in reality *A. centripunctata*. In the case of the species described by Gough, I have shown that this is certainly distinct from the type species, and I re-named it *A. goughi* (Woodland 1927) ; I have also described an *Avitellina* species from India—*A. lahorea*—which is equally distinct from the type species. As regards sheep in other parts of Africa, I have described *A. sudanea* and *A. chalmersi* from the Anglo-Egyptian Sudan, and Nagaty (1929) has described *A. southwelli* from Accra, West Africa.

If, then, there is good reason to doubt whether *A. centripunctata* has really been discovered† in sheep in Africa and India, there is still more doubt as to whether this species has been found in other and non-domesticated ruminants, such as *Hippotragus equinus*, *Cephalopus grimmia*, *Oreotragus oreotragus*, *Taurotragus oryx*, *Aepyceros melampus* and others recorded by Baer (1927) and Mönnig (1928). Some justification for this scepticism is afforded by my description (Woodland, 1928) of *Anootypus edifontaineus* from *Taurotragus oryx* (and *A. ricardi* from *Cobus* sp.), by Nagaty's (1929) description of *Avitellina aegyptiaca*

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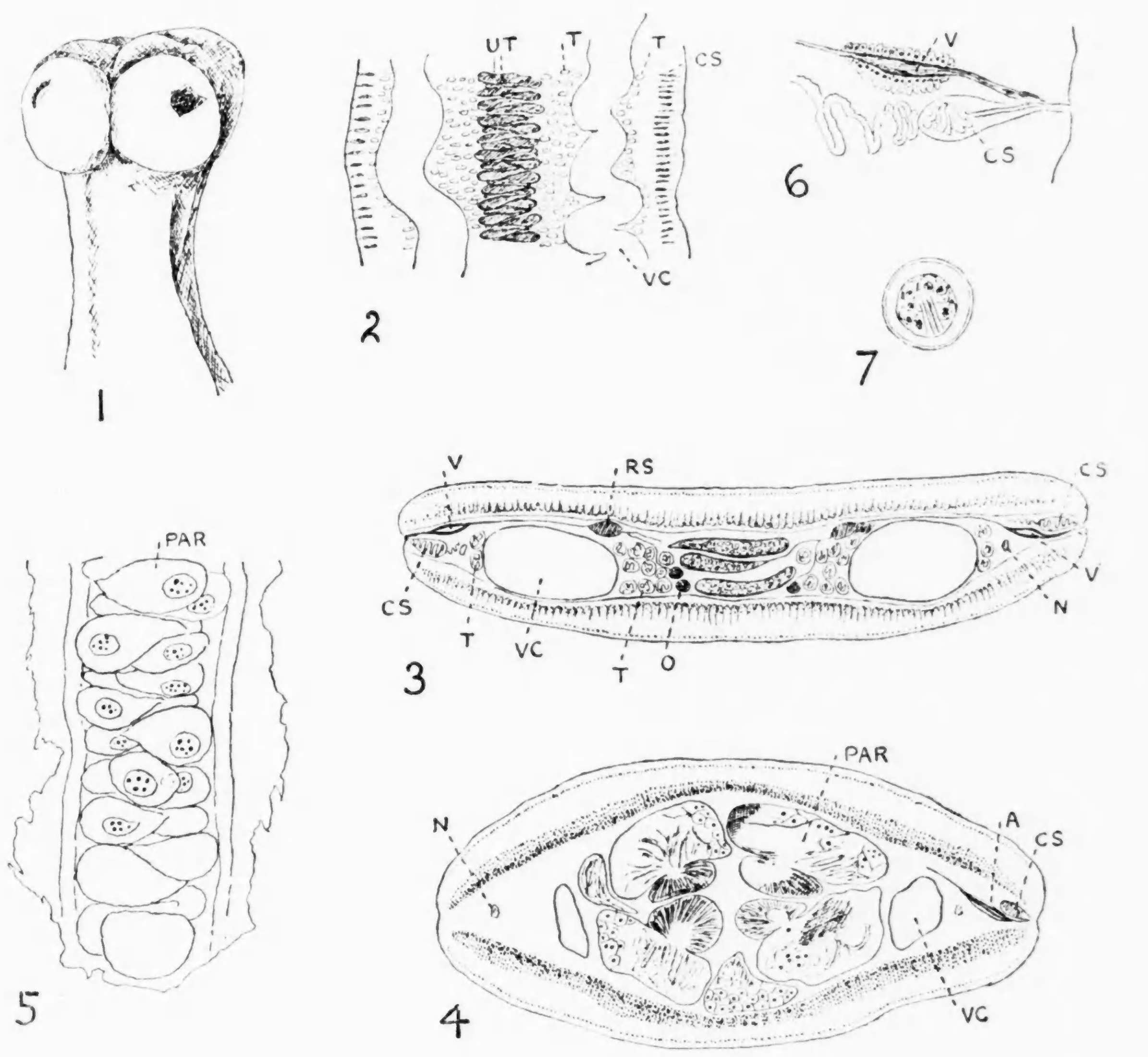
*And in the ox from Freetown, West Africa, by Nagaty (1929). I doubt, however, if the worm in this host is identical with *A. centripunctata*, since, although similar, there are distinct differences, as, for example, the absence in the former species of inter-segmental septa, the small size of the ovaries (only 50μ compared with 120μ), the slightly alternating arrangement of the uteri even in young male-mature proglottids, the absence of egg-capsules, and the small size of the ventral excretory canals and paruterine organs in gravid proglottids.

†I do not say that this species does not occur in Africa and India (it may have been imported), but only that statements to that effect have not yet established the fact.

from *Cephalops* sp. in North-Eastern Rhodesia, and more recently by Fuhrmann's (1933) account of *Anootypus monardi*, also from *Taurotragus oryx*. Thanks to Dr. Sandground, who kindly supplied my material, I am now able to prove in one further instance that a worm, parasitic in *Hippotragus equinus* at a place called Ebombo in the North Katanga district of the Belgian Congo, and labelled '*Avitellina ? centripunctata*' in the collection of the Zoological Museum of Harvard University, is quite distinct from the type species *A. centripunctata*, though superficial examination of the entire specimen might not detect the fact.

The single fragmented tapeworm placed at my disposal measured (in formalin) approximately 40 cm. in length, with a maximum breadth (male-mature region) of 3·42 mm. (slightly more than normal, since the strobila is contracted longitudinally). The principal feature of the strobila is the extreme shortness of the segments, even as compared with most other species of *Avitellina*, and this is accentuated in the present specimen by the longitudinal contraction mentioned (shown by the sinuous form of the excretory canals). Even in a fairly well extended (longitudinally) region there are about 130 cirrus sacs in 4·7 mm. of strobila, which means that the average segment in this region is only 0·036 mm. long for a breadth of 3 mm. The result of this shortness of the segments is that relatively bulky bodies like the paruterine organs are very much crowded, and when these attain their maximum size as many as six (some only represented by small pieces) may be visible in a transverse section 10μ thick (fig. 4). Even in the male-mature region, adjacent uteri lie in different planes as a preparation for the crowding to come (fig. 3). The scolex (fig. 1) is similar to that of *Avitellina centripunctata* both in form and size (2·06 mm. broad and 1·29 mm. long, from the base of the suckers to the apex). The external diameter of the suckers is about 0·88 mm., and in this specimen the openings are very contracted.

The general structure of the strobila is typically Avitellinine. The testes (fig. 2) are disposed in the usual four columns (1–2 *wide*, i.e., two testes in a *transverse* row, in the two outer columns, and 3–5 wide in the two inner columns), but differ from those of the type species in the columns being 2–4 *deep dorso-ventrally*; indeed, in contracted regions of the strobila the testes in transverse sections form closely packed masses on each side of each ventral excretory canal, and in the young male-mature strobila the testes in the outer columns are 2–4 wide. The fully developed testes measure on the average in transverse sections about 0·087 mm. in diameter (in *A. centripunctata* the testes measure about 0·062 mm.), are slightly compressed (0·066 mm.) antero-posteriorly, and apparently fit into the short segments by the testes of two consecutive segments becoming pressed into a single transverse row. The cirrus sacs are, as is typical of *Avitellina* species, situated, in the vast majority of cases, dorsal to the vulvae (the distended portions of the vaginae situated next to the external openings) on the right side of the strobila and ventral on the left side. The cirrus sacs measure in transverse sections about 0·117 mm. long and 0·073 mm. maximum breadth, and contain a much convoluted ductus. The cirrus is thin and unarmed and



Avitellina sandgroundi sp. nov.

FIG. 1. The scolex, sketched in formalin. × 12.
F G. 2. The male-mature region of the strobila. × 12.
FIG. 3. Transverse section of the male-mature region of the strobila, drawn from a section 30μ in thickness (slightly diagrammatic). × 27.
FIG. 4. Transverse section of the strobila in the gravid region. × 27.
FIG. 5. Outline sketch of end of strobila showing the empty paruterine organs. × 27.
FIG. 6. The cirrus sac and vulva in a transverse section. × 87.
FIG. 7. An egg, sketched in balsam. × 530.

Reference letters of figures

CS = cirrus sac.
N = nerve.
O = ovary.
PAR = paruterine organ.
RS = receptaculum seminis.
T = testis.
UT = uterus.
V = vulva of vagina.
VC = ventral excretory canal.

was not extruded in my preparations. The vulvae are about 0·219 mm. long and 0·022 mm. in maximum breadth (not including the gland cell layer which invests the broadest region), and are thus nearly twice the length of the cirrus sacs (fig. 6), as in *A. centripunctata*. Unlike those in *A. centripunctata*, the sacs and the vulvae lie in the same dorso-ventral plane—the former are not anterior to the latter. The portion of vagina internal to the vulva is very slender and opens into the receptaculum seminis (0·164 × 0·054 mm. in transverse sections) situated above the internal wall of the large ventral excretory canal. The connections with the ovary and the uterus are as in *A. centripunctata*, and the paruterine organs are of the usual type, though crowded into six rows (fig. 4). The ovary is very small (about 0·076 × 0·047 mm. in transverse sections)—appreciably smaller than the testes (the reverse is the case in *A. centripunctata*). The eggs (fig. 7) contained in the capsules formed in the fully developed paruterine organs are 18–20μ in external diameter, and the embryo is 10–11μ. The hooks are about 5μ in length. I cannot detect an inner shell. I can find no clear evidence of the egg-capsules and loose eggs in the paruterine organs being discharged into the ventral excretory canals ; on the contrary, the excretory canals become distinctly smaller in the gravid region of the strobila, and the capsules and eggs appear to be freed by the rupture of the paruterine organs at the end of the strobila. My material plainly shows empty paruterine organs and a general disintegration of the strobilar tissues in the terminal segments (fig. 5). The longitudinal muscle system consists of a single layer of large bundles of fibres demarcating the medulla dorsally and ventrally, and a thin layer, one fibre deep, underlying the cuticle. There are not two layers internal to the subcuticula, such as exist in *A. centripunctata*, in addition to the thin layer underlying the cuticle. I have been unable to detect small dorsal excretory canals in my sections, but the ventral canals are of course very large but diminish in size in gravid proglottids and evidently do not serve as egg-ducts, as in the type species.

That this *Avitellina* species from *Hippotragus equinus* is not *A. centripunctata* is, I think, proved by the differences noted above, as, for example, the extreme shortness of the segments and the consequent crowding of the testes, ovaries and uterine organs, the alternate arrangement of the uteri even in young male-mature segments, the larger testes and very small ovaries, the smaller eggs and the discharge of these directly to the exterior instead of into the ventral canals, and the situation of the cirrus sacs in the same dorso-ventral plane as the vulvae. On the other hand, this species does undoubtedly show some resemblances to *A. southwelli*, parasitic in sheep at Accra, West Africa, and described by Nagaty (1929). The dimensions of the testes, ovaries, cirrus sacs, vulvae and eggs are roughly the same in the two cases, in both the uteri have from the first an alternate arrangement, and the sacs and vulvae lie in the same dorso-ventral plane. But the facts that the eggs in *A. southwelli* are discharged into the ventral excretory canals, as in *A. centripunctata*, that the testes are at most and only occasionally 2-deep dorso-ventrally, and that testes are present external to the

nerve cords, and the relatively long segments compared with those above described (in *A. southwelli* ' the uteri lie in the middle fifth in the male-mature strobila and the middle third in gravid strobila '), all point to the two species being distinct. I propose to name this new species *Avitellina sandgroundi*, after Dr. Sandground, the donor of the material. The type material is deposited in the Museum of Comparative Zoology, Harvard University.

The definition of the genus *Avitellina* (Woodland, 1927, p. 406) must be amended by omitting the statement that the cirrus sacs are always anterior to the vulvae. On the other hand, Southwell (1929) and Nagaty (1929) are clearly unaware of the relative constancy and therefore importance of the character that in the vast majority of cases the cirrus sacs of the right side of the strobila are dorsal to the vulvae and ventral on the left side. In the worm described above, for example, sagittal sections through a portion of strobila containing fifty segments do not show a single exception to this rule, and this rule has been shown to hold, not only for the type species, but also for *Avitellina goughi*, *A. lahorea*, *A. sudanea* and *A. chalmersi*. I strongly suspect that it also holds for *A. southwelli* and *A. aegyptiaca*. Southwell's simple statement (as part of the defination of the genus) that in *Avitellina* the cirrus sacs are either dorsal or ventral to the vulvae does not, in my opinion, do justice to the importance of this peculiar feature.

REFERENCES

Many of the references cited are given in :—

WOODLAND, W. N. F. (1927). On three new species of *Avitellina* (Cestoda) from India and the Anglo-Egyptian Sudan, with a re-description of the type-species *A. centripunctata* (Rivolta), 1874. *Ann. Trop. Med. & Parasitol.*, **21**, 385.

The following are references not contained in that paper :—

BAER, J. G. (1927). Monographie des cestodes de la famille des Anoplocephalidae. *Bull. Biol. France et Belg.*, suppl. X.

BHALERAO, G. D. (1934). The common worms of sheep and goats in India and their control. *Agric. Live-Stk. India*, **4**, 655.

FUHRMANN, O. (1933). Deux nouveaux cestodes de mammifères d'Angola. *Bull. Soc. Neuchâtel. Sci. Nat.*, **58**, 97.

MÖNNIG, H. O. (1928). Check list of the worm parasites of domesticated animals in South Africa. *13th & 14th Rep. Vet. Res. S. Afr.*, pt. II, 801.

NAGATY, H. F. (1929). An account of the anatomy of certain cestodes belonging to the genera *Stilesia* and *Avitellina*. *Ann. Trop. Med. & Parasitol.*, **23**, 349.

SOUTHWELL, T. (1929). Notes on the anatomy of *Stilesia hepatica*, and on the genera of the sub-family *Thysanosominae* (including *Avitellininae*). *Ibid.*, **23**, 47.

VEGLIA, F. (1919). Il vermi parasitti negli animali del Sud-Africa. *Ann. Accad. Agric. Torino*, **62**, 16.

WOODLAND, W. N. F. (1928). On a new genus of Avitellinine tapeworms from ruminants in East Africa. *Parasitol.*, **20**, 56.

OBSERVATIONS ON LEISHMANIASIS IN THE DISTRICT OF CANEA (CRETE)

BY

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(*Received for publication* 24 *April*, 1935)

The island of Crete is to-day one of the most important foci of both visceral and cutaneous leishmaniasis of Greece and probably of the whole Mediterranean basin.

Archer (1907), of the British army detachment in Crete, appears to be the first to have diagnosed and treated a case of visceral leishmaniasis in Crete (in a British soldier). Since then many cases have been observed, but it is only in recent years that the extent of the infection has been realized.

From 1923 to 1929 cases were treated in the district dispensary of Canea, but no exact statistics were made. During this period 50 cases of visceral leishmaniasis were observed, mostly in children 1 to 10 years old ; they all showed typical symptoms—fever, splenomegaly, anaemia, and in a few severe cases also petechiae of the skin and mucous membranes.

More reliable data were obtained in 1930 and in subsequent years, owing to the establishment of a Health Centre with a dispensary for visceral leishmaniasis, where parents were encouraged to bring children for treatment.

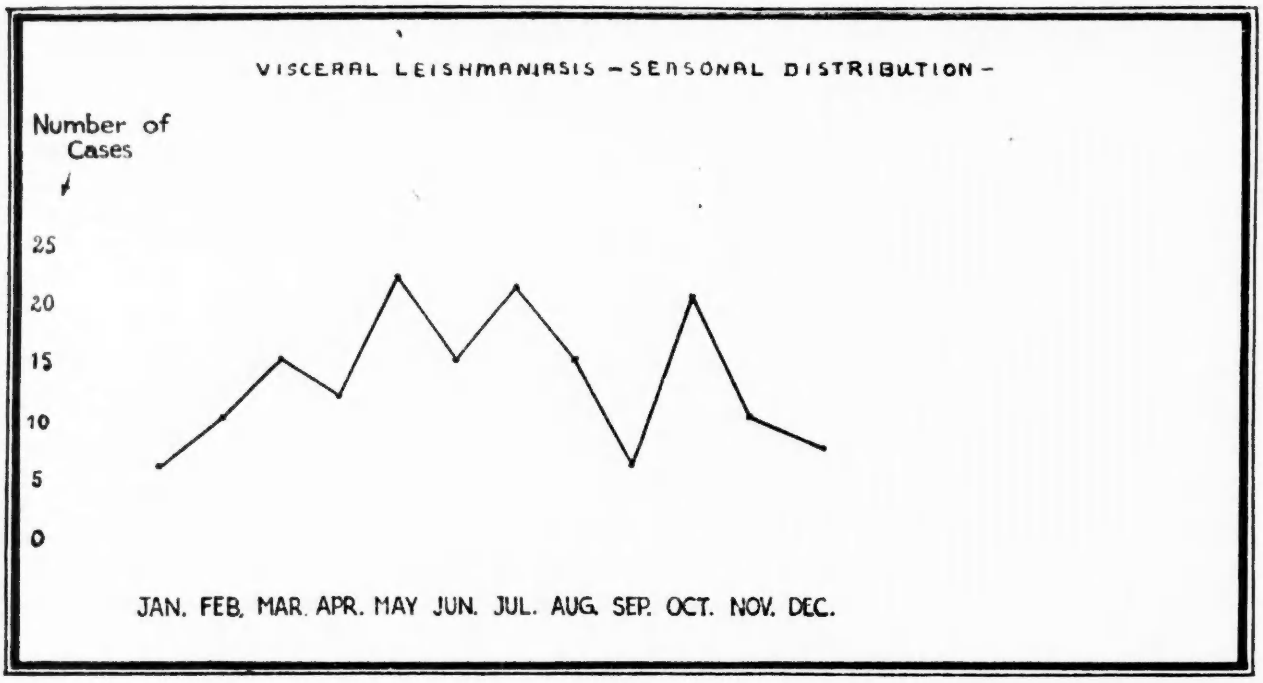
Most of our cases came from the town of Canea (population 30,000), only a few coming from the outlying villages. It must be emphasized that our statistics are based on the number of cases treated in the Health Centre ; they do not include cases treated by private physicians or cases wrongly diagnosed. They do not, therefore, give an adequate picture of the intensity of the infection in Canea.

During 1930, only 12 cases were observed ; a further 160 cases were noted from 1931 to 1934. The small number observed before 1931 is not due to an increase in the number of cases during the last four years, but to the fact that in the previous years a number of cases were overlooked. The true incidence of visceral leishmaniasis is not yet known, but the number of cases diagnosed is increasing annually owing to improved laboratory facilities, the increase in the number of outpatients at the dispensary of the Health Centre, and the fact that private physicians are paying more attention to the disease. Including the cases treated by private physicians, there are, at a very conservative estimate, no less than 50 cases per annum in the district of Canea.

All cases are diagnosed by examination of smears from spleen punctures. In addition, Napier's formol-gel reaction was used in the last 18 cases. The reaction was positive in all cases, including one in which a spleen smear was negative during the first examination. On Professor Adler's recommendation,

the formol-gel reaction was performed with defibrinated blood as well with serum for a control. Six cases were examined and the reaction was positive ; the gelification was rather more rapid with the defibrinated blood than with serum. Should this observation be confirmed in more cases, the formol-gel reaction with defibrinated blood will be of considerable value because of its simplicity, the small amount of blood required and its applicability in remote villages without laboratory facilities.

Seasonal Incidence. Cases occur throughout the whole year but are fewest in the winter months. It is not possible, on the basis of our figures, to give an accurate picture of the seasonal distribution, for some cases are diagnosed a few



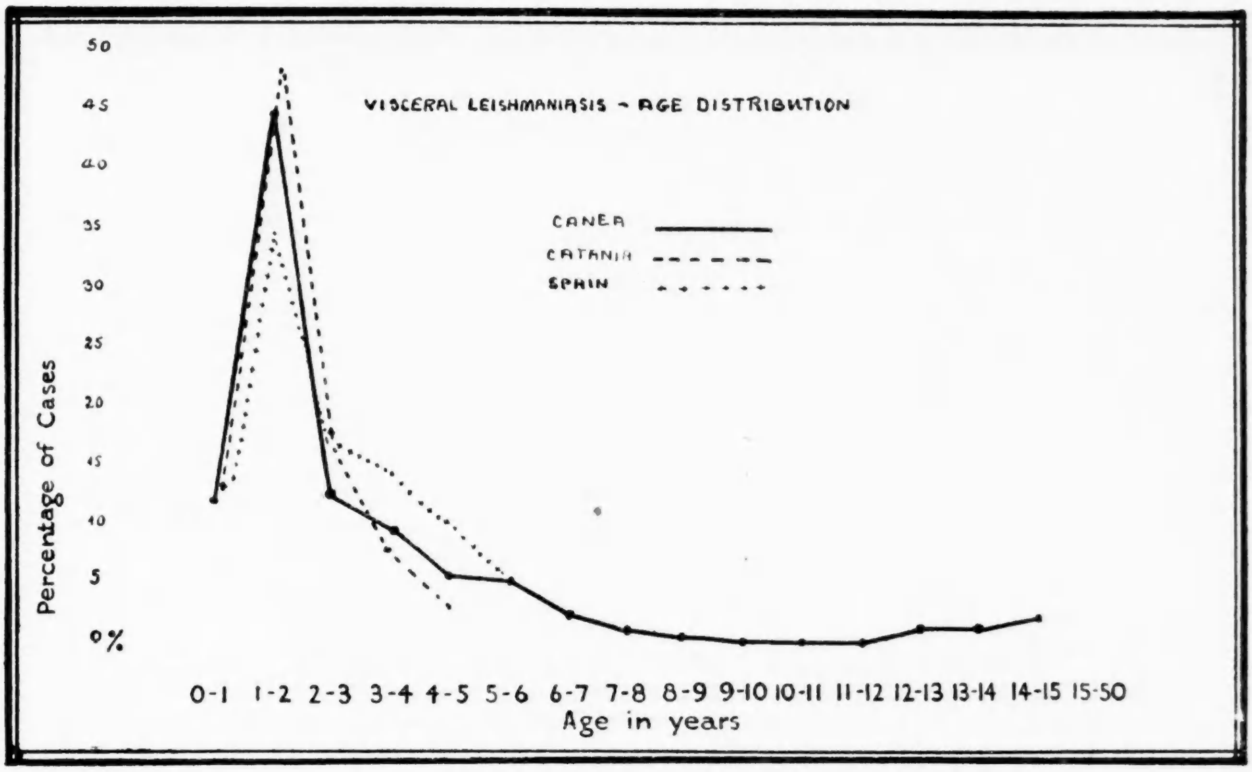
GRAPH. 1.

weeks after appearance of symptoms and others only after months of treatment as chronic malaria. Graph 1 shows the distribution for the years 1930-34 ; for the reason stated above, its value is limited.

Age Incidence. The age incidence is shown in Graph 2, and for comparison the age incidence in Catania (from figures compiled by Paradiso, 1926) and in Spain (from figures compiled by Pittaluga, 1912-24) are shown. In all three cases the age distribution is somewhat similar. The disease is mainly one of infancy : both in Italy and Greece fully 50 per cent. of all cases occur in infants under two years of age. One or two cases annually are found in adults.

Local Distribution. The local distribution is shown in the plan of Canea. The disease is localized in a few quarters on the periphery of the town, and infected houses usually have gardens or court-yards. The majority of the cases

come from Hagios Ioannis, which was built during the last ten to fifteen years. It is to be noted that there are very few cases in the old town, and these were possibly contracted in other parts of the town. There is a distance of only 350 metres between the endemic focus of Koun-Kapi and the old town, which is practically free from the disease, and a distance of a little more than 1 kilometre



GRAPH 2.

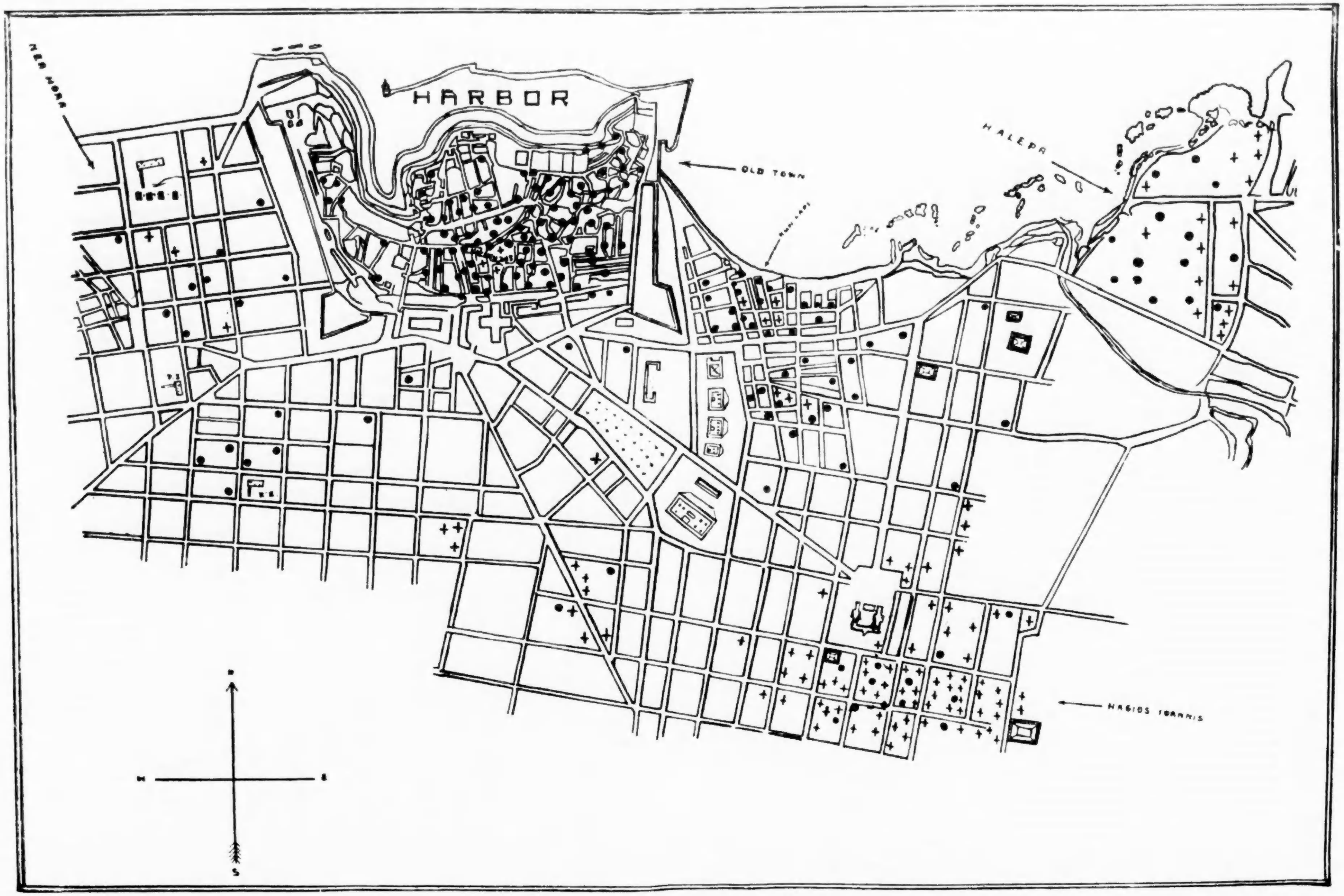
from the relatively intense focus of Hagios Ioannis and the old town. The endemic areas do not differ from the non-endemic areas in the economic or social status of their inhabitants.

Adler and Theodor (1932), on the basis of a study of the sand-flies in Greece, suggested that *Phlebotomus major* is the main carrier in Greece. Adler, Theodor and Witenberg made a study of the sand-flies of Canea during 1934, and their results await publication.

ASSOCIATION OF CANINE AND HUMAN VISCERAL LEISHMANIASIS

Parents were invariably questioned as to the association and condition of dogs in their immediate neighbourhood. Many cases reported the presence of sick dogs in or near their houses, and in a few cases the parents stated that they had destroyed their dog because of sickness and emaciation.

It is to be noted that, owing to a rabies scare in 1933, there was a wholesale destruction of street dogs which commenced in May, 1933. As a result, street



Plan of Canea. Scale 1 : 5000. + Houses with cases of visceral leishmaniasis. ● Houses with cases of cutaneous leishmaniasis.

dogs were very rare in Canea during 1934. There were 30 cases of infantile leishmaniasis from Canea treated at the Health Centre during 1934, as against 42 during 1933 and 45 during 1932. During 1934 a thorough search for cases of infantile leishmaniasis was constituted throughout Canea, and it is therefore probable that in contrast to previous years very few cases escaped observation during 1934. It is concluded that the wholesale destruction of street dogs had an appreciable effect on the incidence of the disease in children.

Treatment. There is nothing exceptional to report, and our results correspond to that of other observers in Mediterranean countries (e.g., Zahra-Neumann, 1933). In 1924, stibenyl and stibosan were tried without satisfactory results. These drugs were not well tolerated and often caused diarrhoea and vomiting. In 1925, neostibosan was introduced in doses of 0·025 to 0·05 gm. intravenously in infants under one year ; injections were repeated every second day, and the dose was gradually raised up to 0·20 gm., treatment being continued till symptoms disappeared. The long duration of treatment was inconvenient, and some cases became absolutely resistant to neostibosan. During the last two years the treatment was modified ; larger doses (up to 0·30 gm. for a child and 0·45 gm. for an adult as a maximum dose) were employed, and the injections were repeated daily. The results improved, fewer cases became resistant, and the duration of treatment was shortened. It is to be noted that two cases which became absolutely resistant to neostibosan were cured by urea-stibamine.

ORIENTAL SORE

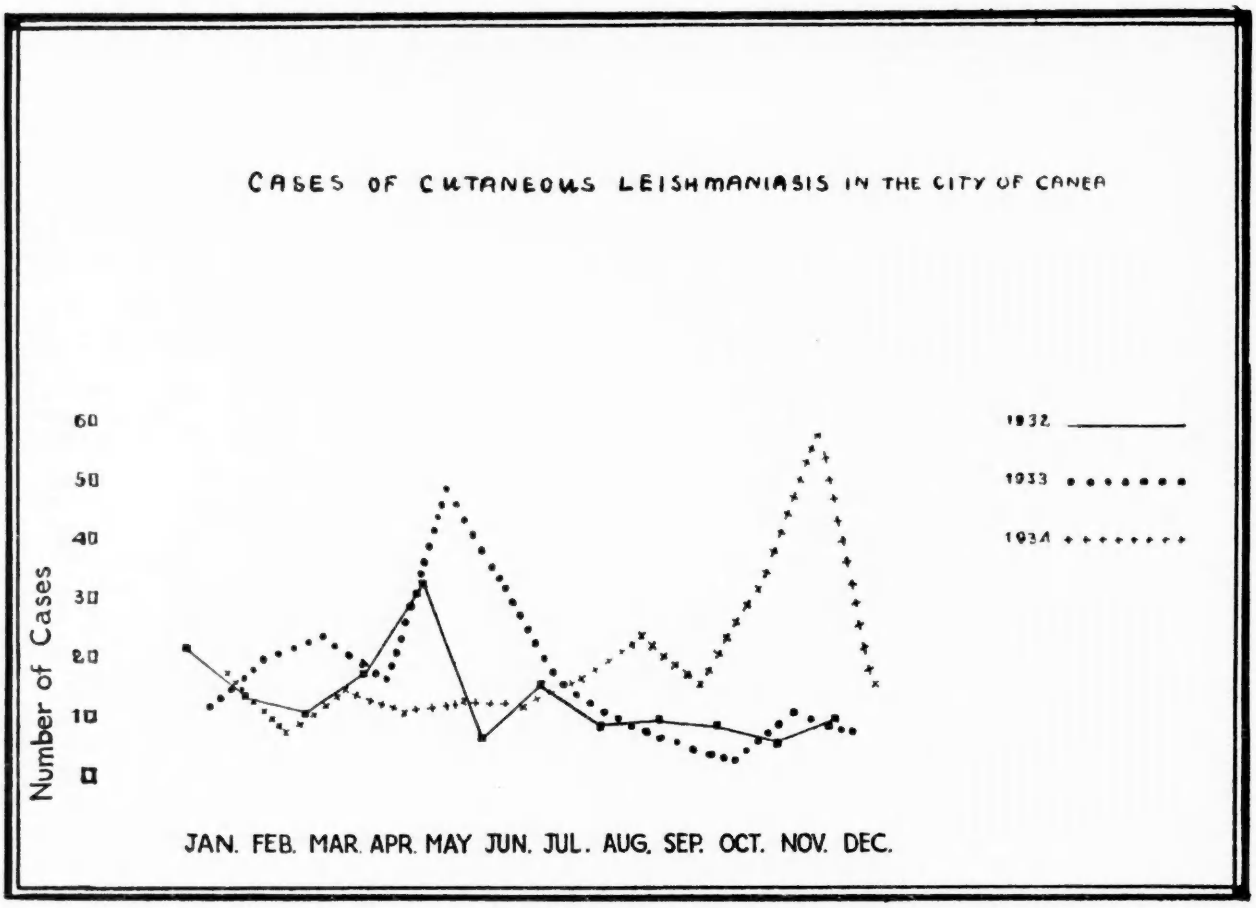
Oriental sore is very common in Crete, where it is known as ' Khaniotiko,' owing to its popular association with the City of Canea (' Khania '). For several years the disease was very common, and boils or typical scars on the face were a prominent feature in the streets of Canea. Between 1932 and 1934 only 614 cases were registered in the dermatological service of the Health Centre, but this number represents only a portion of the actual cases, for many were treated privately and others waited for spontaneous cure without treatment.

The disease as seen in Canea does not present any special features not familiar from the literature. Only one case is of particular interest, for it showed more than a hundred lesions both in parts exposed and not exposed to insect bites. A similar case from Baghdad was reported by Owen (1927). In our case, the multiple lesions probably developed metastatically from an original lesion produced by a sand-fly.

All ages, from infancy to old age, are susceptible to the disease. The local distribution is of particular interest, for it is quite different from that of visceral leishmaniasis. In fact, oriental sore is commonest in the old Turkish town near the harbour, where visceral leishmaniasis is absent. The old Turkish town is mediaeval in construction, with very narrow streets. There are no gardens, the houses are damp and there is ample opportunity for the breeding of sand-flies in stables and deep cracks in the moisture-laden stone walls. Adler (1934)

explains the difference in distribution of the two diseases on the basis of differences in the distribution of various species of the local sand-flies. The seasonal distribution is shown in Graph 3; the variations in successive years are probably due mainly to the fact that patients come for treatment in various stages of the disease.

Treatment. Various methods were tried: infiltration with emetine, injection of antimony compounds, and cauterization by chemical agents. Treatment with injection of neostibosan is lengthy, expensive and gives uncertain results. As a routine treatment in the Health Centre, the application of a suspension of



GRAPH 3.

10 per cent. pulverized vegetable charcoal in concentrated sulphuric acid is used. This treatment was originally introduced for oriental sore by Dr. M. Logiades, district physician in Candia. This method is drastic but well tolerated. The mixture, a thick black fluid, is painted on and a little beyond the lesion, irrespective of the stage of development of the latter. The treatment is repeated two or three times every third day. A thick black crust is formed, which falls in 10 to 14 days. If granulations are left after the crust has fallen, the treatment is repeated. In advanced lesions a scar is left, but cases treated early show practically no scar. This method has the great advantage that no

cases are resistant. The application causes a slight burning sensation, which passes quickly and is in any case less painful than infiltration with emetine.

I have to thank Professor S. Adler and Dr. Witenberg for advice in preparing this paper.

REFERENCES

ADLER, S. (1934). Lecture on the present status of the knowledge of leishmaniases. *Iatrikai Athinai*, no. 45, p. 696. (Report by N. Lorandos in Greek.)

——— and THEODOR, O. (1932). Vectors of Mediterranean kala azar. [correspondence.] *Nature*, **130**, 507.

——— ——— (1935). Investigations on Mediterranean kala azar. VIII : Further observations on Mediterranean sandflies. *Proc. Roy. Soc., Ser. B.*, **116**, 505.

ARCHER, G. J. S. (1907*a*). A case of kala-azar contracted in Crete. *Jl. Roy. Army Med. Corps*, **9**, 287.

——— (1907*b*). Notes on the *post-mortem* examination of a case of kala-azar contracted in Crete. *Ibid.*, **9**, 511.

OWEN, D. U. (1927). A case of oriental sore. *Ann. Trop. Med. & Parasitol.*, **21**, 277.

PARADISO, F. (1926). Sulla distribuzione altimetrica del kala-azar infantile in provincia di Catania e sulla età dei piccoli leishmaniotici. *Pediatria*, **34**, 664.

PITTALUGA, G. (1925). Étude epidémiologique sur la leishmaniose viscerale en Espagne. *Soc. Nat. Geneva.*

ZAHRA-NEUMANN, C. (1933). A report on the treatment of infantile kala-azar. *Trans. Roy. Soc Trop. Med. & Hyg.*, **26**, 383.

STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE GENERA OF THE FAMILY MUSCIDAE TESTACEAE ROBINEAU-DESVOIDY BASED ON A COMPARATIVE STUDY OF THE MALE AND FEMALE TERMINALIA

THE GENERA ADICHOSIA SURCOUF AND AUCHMEROMYIA BRAUER AND VON BERGENSTAMM (sens. lat.)

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In 1830, Robineau-Desvoidy erected the family Muscidae Testaceae, in which he included four genera of yellow muscids, *Bengalia*, *Phumosia*, *Ormia* and *Palpostoma*. In 1835, Macquart sank Robineau-Desvoidy's four genera under the name *Ochromyia*, dividing it into four sections which correspond very well with Robineau-Desvoidy's above four genera. In 1920, Surcouf revised the family, clearing up many doubtful points regarding Macquart's species, to the types of most of which, as well as to those of older authors, he had access. Surcouf re-arranged the species in four groups erecting several new genera as follows :—

Group 1. Genus *Bengalia* R-D.
„ 2. Genus *Phumosia* R-D.
„ *Caiusa* Surcouf
„ *Hemilucilia* Brauer and Bergenstamm
„ *Psilostoma* Surcouf
„ *Chloroprocta* van der Wulp
„ 3. Genus *Chopardia* Surcouf
„ *Ochromyia* Macq.
„ *Mesembrinella* Giglio Tos.
„ 4. Genus *Adichosia* Surcouf
„ *Paratricyclea* Vill.
„ *Cordylobia* Grünberg
„ *Hemigymnochaeta* Corti
„ *Auchmeromyia* Brauer and Bergenstamm
„ *Chœromyia* Roubaud
„ *Zonochra* Brauer and Bergenstamm
„ *Tricyclea* van der Wulp
„ 5. Genus *Amphibolosia* Surcouf
„ *Proekon* Surcouf

In 1920, Villeneuve criticised Surcouf's revision, pointing out that his genera were based on thoracic bristles which are only of relative and insignificant value, and that he had omitted to study the terminalia. Surcouf in reply regrets that he did not examine the terminalia, and repeats the old statement that the characters of the terminalia are only of use in the ♂, mentioning *Sarcophaga* as an example. We know now, however, that this statement is no longer true, and that the ♀ terminalia are just as important as those of the ♂ in establishing the true affinities and relationships of species and genera and in understanding changes in the ♂ parts. This has been amply proved in my recent papers, and will be particularly so in the present one. It is indeed most unfortunate that Surcouf did not study the terminalia of the species of the many genera which he includes in the Muscidae Testaceae, for had he done so he would quickly have discovered, as I shall show in the course of my revision, that many of the genera which he groups together are unrelated, and that some are unnecessary. In 1922, I examined the material on which Surcouf based his revision, and at that time came to the same conclusion ; but I was then unable to prove my contention, which I am now able to do.

The revision which I propose of the genera that Surcouf groups together in the Muscidae Testaceae will be based on a comparative study of the ♂ and ♀ terminalia of many of the available species, and will aim at settling their true affinities and relationships. In dealing with the genera, I shall not follow any particular order, but I shall begin with group 4, in which Surcouf includes eight genera, as noted above. I do not propose to give his definition of the group, as the characters which he enumerates have no phylogenetic significance. In this paper I shall deal with the genera *Adichosia* and *Auchmeromyia*.

Genus **Adichosia** Surcouf. This genus was erected for the well-known reddish-brown blow-fly of Australia, *Calliphora ochracea* Schiner (*hyalipennis* Macq. preoccupied, as noted by Hardy), which had previously been made the genotype of *Neocalliphora* Brauer and Bergenstamm. The Tasmanian form, which Malloch has named *nigrithorax*, is according to Hardy a distinct species. It is possible that Surcouf made it the type of his genus *Amphibolosia*, but one cannot be certain of this as the genus is based on a single ♀ from Tasmania.

In another paper (1935) in this journal I have described and fully illustrated the ♂ and ♀ terminalia of the Australian *C. ochracea*, proving that it is very closely related to *C. erythrocephala*, *vomitoria*, etc. The genera *Adichosia* and *Neocalliphora* must, therefore, sink as synonyms of *Calliphora*. Furthermore, the genus *Calliphora* cannot be placed in a group with the genus *Auchmeromyia*.

Genus **Auchmeromyia** B. and B. This genus at present contains five species, viz., *luteola* F., *chœrophaga* Roubaud, *bequaerti* Roubaud, *boueti* Roubaud and *prægrandis* Austen, of which the larvae are blood-suckers—a fact which is well known, particularly from Professor Roubaud's (1914) valuable monograph. The larva of *luteola* (Congo floor-maggot) feeds on the blood of man, and that of each of the four other species on the blood of Aard Varks (*Orycteropus*) and

wart-hogs (*Phacochoerus*). Roubaud placed the last four species in the subgenus *Chœromyia*. He has made an exhaustive and most important study of the biology, etc., of the species, and I shall refer later to some of his observations.

In dealing with the species I shall in each case describe shortly the diagnostic characters of the adults and of the terminalia, give a key to the species, make some notes on its systematic position and finally re-define the genus in the light of this new work. I should like to take this opportunity of thanking Professor Roubaud for his generous gift of additional specimens of some of the species and of a larva of *chœrophaga*, and of thanking Mr. W. H. Potts and Mr. A. Cuthbertson for many specimens of *luteola*, *bequaerti* and *prægrandis*; without this material I could not have completed this study. I should like also to thank my wife for the drawings of the abdomens of the species, which, as will be noted, have been drawn with the head directed towards the observer, in which position the markings should be noted. As the terminalia are very fully illustrated, and therefore speak for themselves, the descriptions of the parts are as brief as possible. As already noted in another paper (1935), the sclerites of the ovipositor appear to be quite different in structure in some of the drawings of the whole mounts and in some of each sclerite when dissected off and mounted flat. These discrepancies are simply due to the fact that in the former the sclerites appear foreshortened. In the case of the ovipositor of *luteola*, *chœrophaga*, *bequaerti* and *boueti*, the drawings are made to the same scale, and those of the separate sclerites are on a smaller scale than the same plates in the entire ovipositor; this will explain the differences in size.

Auchmeromyia luteola F. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. A large light-yellow species, the third tergum (apparent second) markedly longer than the fourth in both sexes. ♂. *Head.* Eyes widely separated; vertex about, or slightly less than, width of an eye.

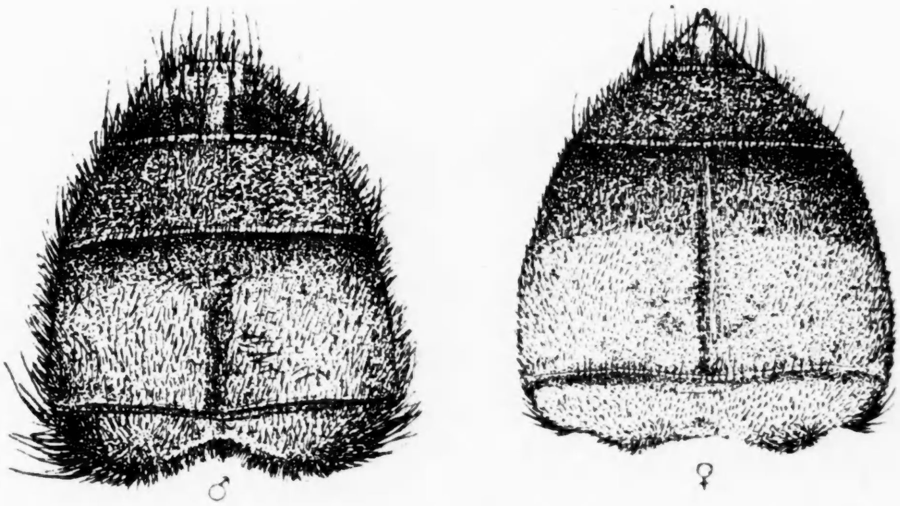

FIG. 1. ♂ and ♀ abdomen of *luteola*.

Thorax. Mesonotum with a pair of dark admedian stripes, part before the suture wide. *Abdomen.* Fig. 1. Terga 1 and 2 (apparent first) yellow ; tergum 3 yellow, with a narrow posterior black band and a median dark stripe, wide posteriorly and narrowing anteriorly, and about one and a half times longer than tergum 4 ; tergum 4 black with white tomentum, except extreme antero-lateral edges and anterior border, both of which are yellow ; one pair or more of strong marginal bristles; tergum 5 about anterior half black, remainder yellow.

♀. *Head.* Eyes widely separated ; vertex about three-quarters width of an eye. *Thorax.* Mesonotum very similar to that of ♂, but stripes as a rule better marked, especially behind suture. *Abdomen.* Fig. 1. Terga 1 and 2 light yellow, sometimes with a faint median stripe and a narrow posterior black band ; tergum 3 about twice the length of tergum 4 ; yellow except about posterior half which is black ; tergum 4 black, extreme posterior edge light yellow with a row or marginal bristles ; tergum 5 triangular-shaped, as seen from dorsal surface, ending in a blunt point (upturned) with a tuft of small

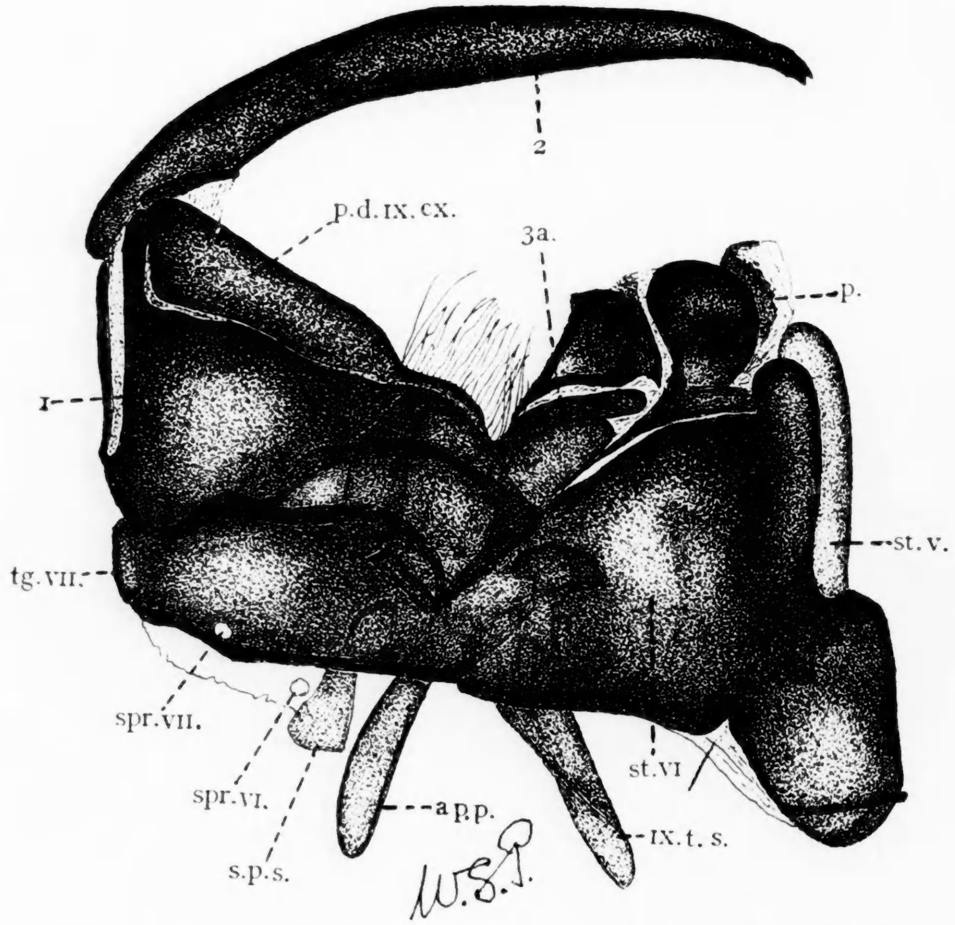

Fig. 2. Seventh and tenth terga, fifth and sixth sterna, anal cerci, ninth coxite and phallosome of *luteola* in side view. *1.*—Tenth tergum ; *2.*—Fused anal cerci ; *3a.*—Distal segment of ninth coxite ; *ap.p.*—Apodeme of phallosome ; *p.*—Phallosome ; *st.v.*, *st.vi.*—Fifth, sixth sterna ; *spr.vi.*, *spr.vii.*—Sixth, seventh spiracle ; *tg.vii.*—Seventh tergum ; *s.p.s.*—Sperm pump sclerite ; *ix.t.s.*—Ninth tergo-sternum ; *p.d.ix. cx.*—Posterior prolongation of distal segment of ninth coxite.

bristles, basal part black, apex light yellow ; ventrally the edges of the tergum lie close together concealing the terminalia (fig. 4).

Salient Diagnostic Characters of Male Terminalia. The ♂ terminalia with anal cerci drawn back are illustrated in side view in fig. 2, and the details of the phallosome, etc., in fig. 3. Sclerites. Sternum 5 as in fig. 3, *b* ; tergum 6 is wanting. Sternum 6 (or apodeme) is a long, broad, irregular plate articulating with the anterior end of tergum 7 on the left and extending round to the right ; it is fused in the middle line in the usual way with the dorsal surface of sternum 5 (fig. 2). Tergum 7 (fig. 2) is very narrow posteriorly and is closely applied to tergum 10. The ninth tergo-sternum is a wide plate, as shown in fig. 3, *c*, the posterior processes articulating with the fused proximal segments of the ninth coxites. Tergum 10 (fig. 2) is long, narrowing posteriorly with a deep wide incision ; the antero-ventral border is closed by the anal cerci and the ventral gap by the posterior prolongations of the distal segments of the ninth coxites (fig. 2). Antero-dorsally tergum 10 ends in a stout plate-like process bent upwards and articulating on each side with the ninth tergo-sternum.

Appendages. *Ninth Coxite.* Lateral view. Fig 3, *h*. *Distal segment.* A rather long narrow finger-like process armed with bristles and hairs on outer side, especially at base, and slightly turned in at end ; each lies ventrally at side of the base of the phallosome ; front view as in fig. 3, *i*. The backward extension of the distal segment noted above is well illustrated in fig. 2. *Proximal Segment.* Lateral view. A short stout rod, fused with its fellow in the middle, as shown in fig. 3, *i*. *Anal Cerci.* Lateral view. Fig. 2. Fused to form a long curved rod bifid at end ; ventral view as in fig. 3, *e*. The fused anal cerci fit into the notch of sternum 5 (fig. 3, *b*).

Phallosome. Lateral view. Fig. 3, *a*. Broad and heavily chitinized (black) ; proximal portion short and wide ; distal portion consisting of two parts, the ventral formed by the short widely expanded struts, ending in sharp upwardly and outwardly directed points ; the dorsal portion extends forwards and has a plate-like expansion with serrated edges, and a narrower basal serrated process directed backwards and downwards ; the wide ejaculatory duct opens at the end (fig. 3, *d*). The two parts are united basally by a characteristic chitinous bar and distally by membrane (fig. 3, *a*). *Posterior Process of Phallosome.* Fig. 3, *a*. Very short. *Apodeme of Phallosome.* Fig. 3, *a*. Long. *Sperm Pump Sclerite.* Fig. 3, *a*. Long. *Parameres.* Lateral view. *Anterior Part.* Fig. 3, *a*, *g*. A small plate ending in a rounded point and armed with long hairs attached along curved outer border. *Posterior Part.* Fig. 3, *a*, *g*. A long, upstanding plate, ending in a bent point, with a long stout hair attached near end on a ridge ; many sensory spines on outer ventral side near end (fig. 3, *f*).

Salient Diagnostic Characters of Female Terminalia. Ovipositor. Segments 6, 7, 9 and 10. The ventral view of the abdomen showing the ovipositor in the position of rest is illustrated in fig. 4, the dotted lines represent the normal position of the ventral edges of tergum 5 ; when treated with caustic

potash they separate. The fully extended ovipositor and the several sclerites are illustrated in fig. 5 ; it will be noted that the ovipositor is wide and of medium length (shorter than that of such genera as *Calliphora*, *Musca*, etc.). As the exact

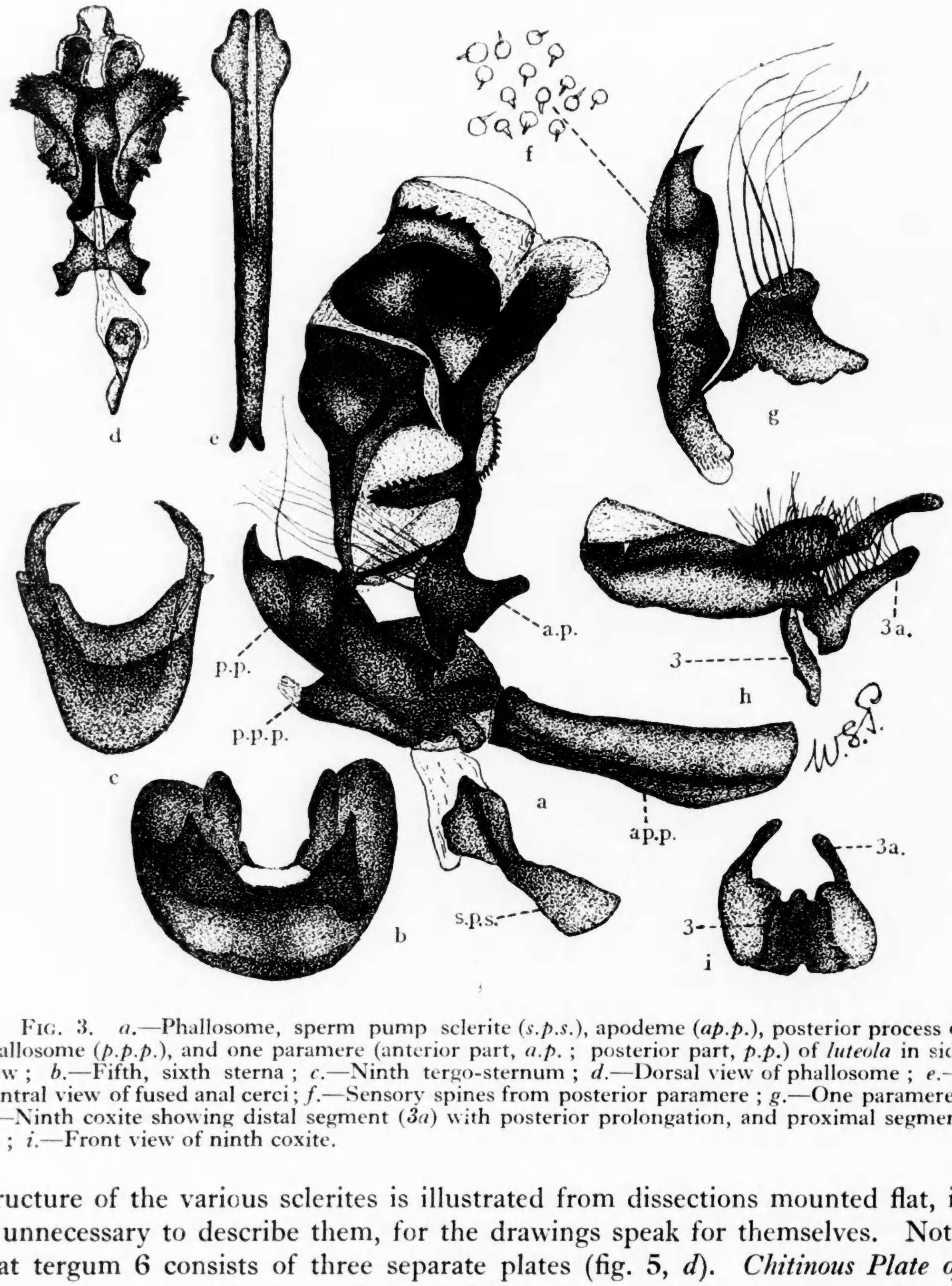

Fig. 3. *a.*—Phallosome, sperm pump sclerite (*s.p.s.*), apodeme (*ap.p.*), posterior process of phallosome (*p.p.p.*), and one paramere (anterior part, *a.p.* ; posterior part, *p.p.*) of *luteola* in side view ; *b.*—Fifth, sixth sterna ; *c.*—Ninth tergo-sternum ; *d.*—Dorsal view of phallosome ; *e.*—Ventral view of fused anal cerci ; *f.*—Sensory spines from posterior paramere ; *g.*—One paramere ; *h.*—Ninth coxite showing distal segment (*3a*) with posterior prolongation, and proximal segment (*3*) ; *i.*—Front view of ninth coxite.

structure of the various sclerites is illustrated from dissections mounted flat, it is unnecessary to describe them, for the drawings speak for themselves. Note that tergum 6 consists of three separate plates (fig. 5, *d*). *Chitinous Plate of Uterus* (signum). Fig. 5, *o*. Large, with two stronger plates. *Spermathecae.*

Fig. 5, *p*. Two long and one short. *Lateral Cutting Plate of Larva*. As in fig. 5, *i*. *Anterior Spiracle*. Fig. 5, *g*. Suggestive of that of *Sarcophaga*, with about 10 small openings. *Posterior Spiracles of Third Larva*. Fig. 5, *i*. Small, widely separated ; breathing slits straight and directed outwards.

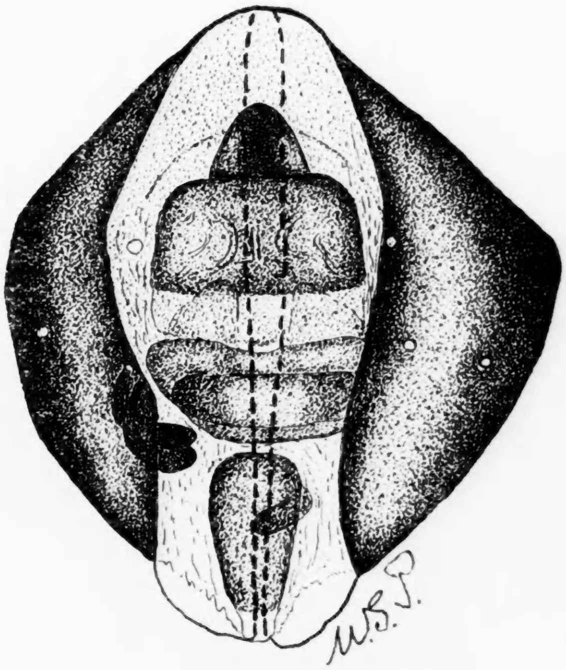

FIG. 4. Ventral view of abdomen of ♀ *luteola* ; the dotted lines show the normal position of ventral edges of tergum 5.

Auchmeromyia bequaerti Roubaud. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF TERMINALIA. A smaller light-yellow species, the abdominal terga not showing any marked inequality in length ; tergum 4 of ♂ with slight, and of ♀ with marked, dorsal incision. ♂. *Head*. Eyes widely separated ; vertex about half width of an eye ; a black spot at lower end of vertex near eye margin. *Thorax*. Mesonotum with a pair of broad black admedian stripes not reaching posterior border. *Abdomen*. Fig. 6, *b*. Terga 1 and 2 yellow ; tergum 3 yellow with a well-marked dark posterior band, which forms in middle a short posterior stripe ; tergum 4 black with white tomentum, except anterior border and sides which are yellowish ; posterior margin slightly emarginated towards middle ; tergum 5 yellow with two large admedian black patches extending to anterior border.

♀. *Head*. Eyes widely separated ; vertex about three-quarters width of an eye, black spot not so well marked. *Thorax*. Mesonotum as in ♂. *Abdomen*. Fig. 6, *a*. Terga 1 and 2 light yellow with a very narrow black posterior dark band ; tergum 3 much as in ♂, but black markings more extensive ; tergum 4 with a marked V-shaped posterior incision, so that tergum in middle line is about half the length of tergum 3 and is almost entirely black ; tergum 5 about

equal to, or a little longer than, tergum 3 ; yellow with a pair of black anterior admedian spots, and sides with some black markings.

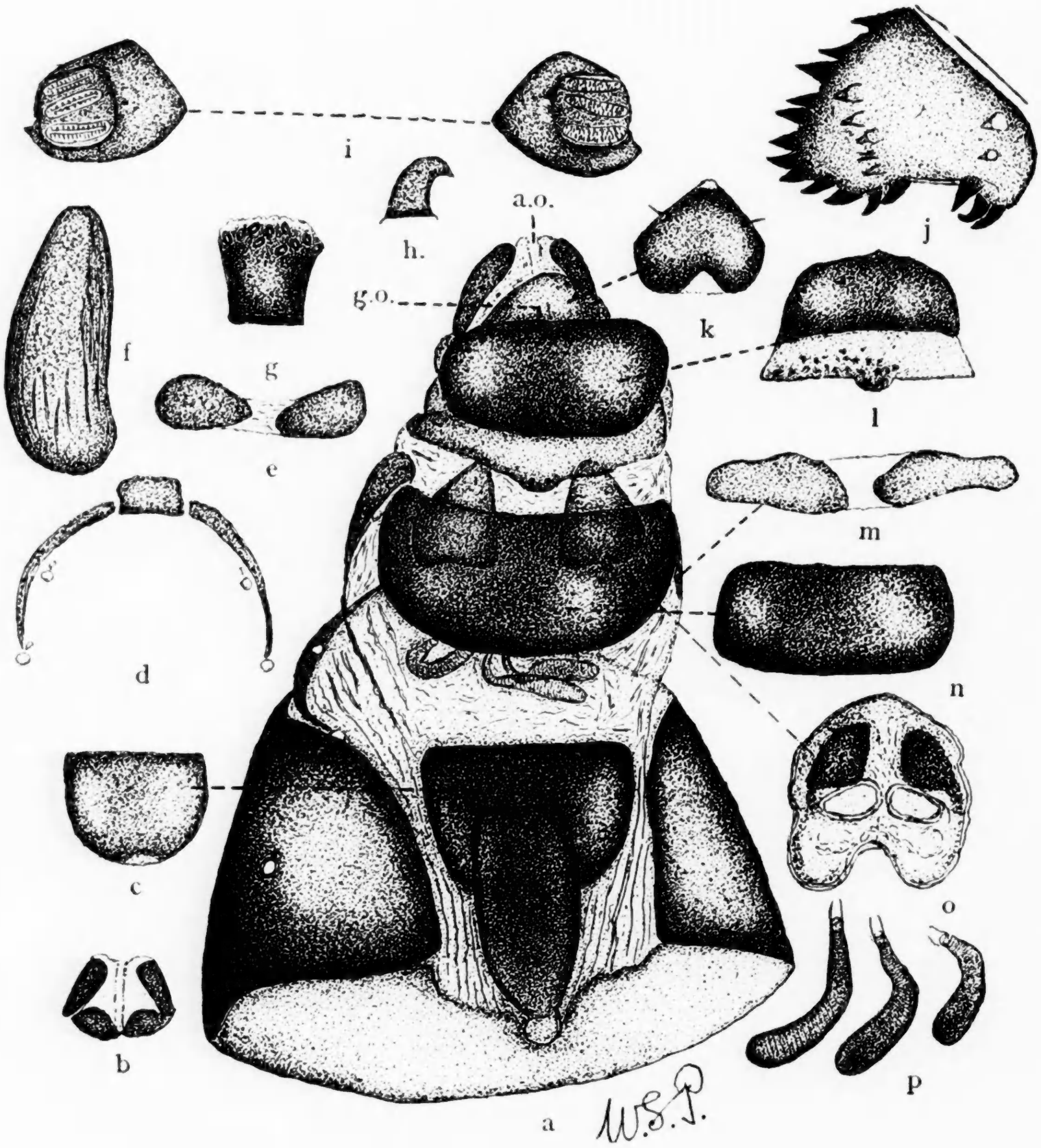

FIG. 5. *a.*—Ventral view of extended ovipositor of *luteola* showing genital opening (*g.o.*), anal opening (*a.o.*) and sclerites ; *b.*—Tenth tergum and anal cerci ; *c.*—Sixth sternum ; *d.*—Sixth tergum ; *e.*—Ninth tergum ; *f.*—Egg ; *g.*—Anterior spiracle ; *h.*—Sensory hook of larva ; *i.*—Posterior spiracles of third larva ; note small round plates ; *j.*—Cutting plate of third larva ; *k.*—Tenth sternum ; *l.*—Ninth sternum ; *m.*—Seventh tergum ; *n.*—Seventh sternum ; *o.*—Signum of uterus ; *p.*—Spermathecae.

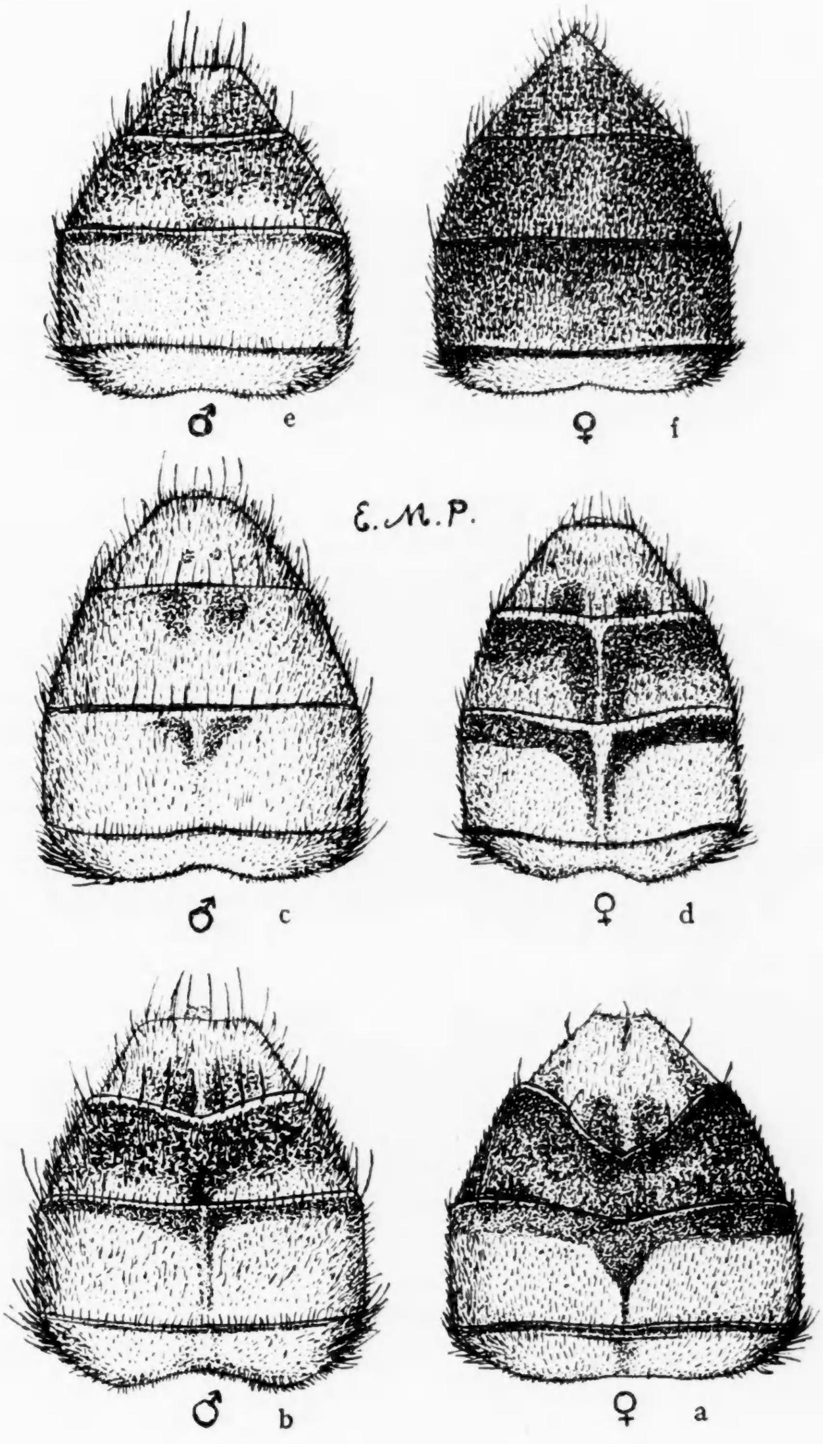

FIG. 6. *a*.—Abdomen of ♀ *bequaerti*; *b*.—Abdomen of ♂ of same; *c*.—Abdomen of ♂ *boueti*; *d*.—Abdomen of ♀ of same; *e*.—Abdomen of ♂ *chœrophaga*; *f*.—Abdomen of ♀ of same.

SALIENT DIAGNOSTIC CHARACTERS OF MALE TERMINALIA. The ♂ terminalia with anal cerci drawn back are illustrated in fig. 7, and the details of the phallosome in fig. 8. SCLERITES. Sternum 5 as in fig. 8, *d* ; tergum 6 is wanting ; terga 7 and 10 as illustrated in fig. 7, which also shows the relation of the fused anal cerci and the posterior prolongations of the distal segment of the ninth coxite to tergum 10. The ninth tergo-sternum is a wide plate, as illustrated in fig. 8, *e*.

APPENDAGES. *Ninth Coxites*. Lateral view. *Distal segment*. Fig. 8, *c*. A short, rather broad, finger-like process turned in at the end and armed with hairs—situated as in *luteola*—and with a similar, but much shorter, posterior

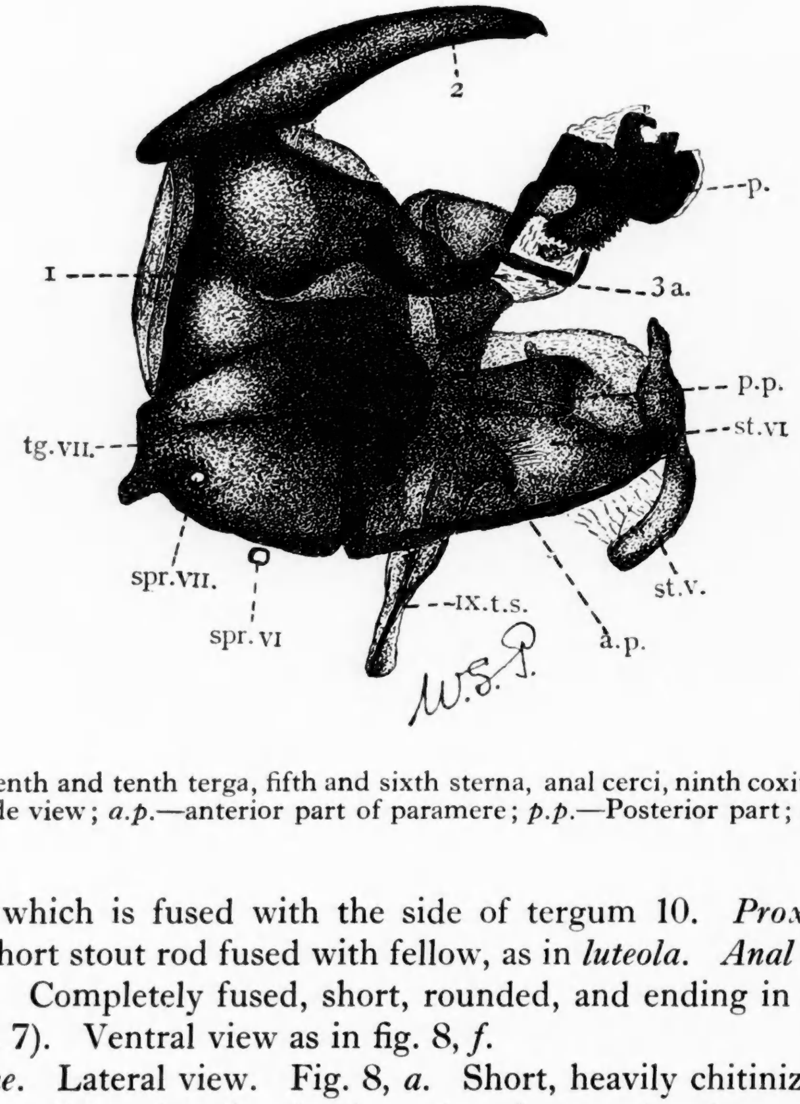

FIG. 7. Seventh and tenth terga, fifth and sixth sterna, anal cerci, ninth coxite and phallosome of *bequaerti* in side view; *a.p.*—anterior part of paramere; *p.p.*—Posterior part; other lettering as in fig. 2.

prolongation, which is fused with the side of tergum 10. *Proximal Segment*. Fig. 8, *c*. A short stout rod fused with fellow, as in *luteola*. *Anal Cerci*. Lateral view. Fig. 7. Completely fused, short, rounded, and ending in a sharp hook-like point (fig. 7). Ventral view as in fig. 8, *f*.

Phallosome. Lateral view. Fig. 8, *a*. Short, heavily chitinized, but not as massive as that of *luteola*. Proximal portion short and rather narrow, bearing the characteristic short posterior process ; distal portion divided into two parts, as in *luteola*, the ventral formed by the struts with a wide lateral serrated flap (fig. 8, *a*) ; distal part consisting of the median rounded shaft bent back at the end to the ejaculatory duct and with a pair of smaller lateral serrated flaps (fig. 8, *g*) ; the two parts united as in *luteola*. *Posterior Process of Phallosome*.

Fig. 8, *a*. Short and narrow. *Apodeme of Phallosome*. Fig. 8, *a*. Long and broad in side view. *Sperm Pump Sclerite*. Fig. 8, *a*. Long and narrow. *Parameres*. Lateral view. *Anterior Part*. Fig. 8, *a*, *h*. Similar to that of *luteola* but smaller and with many more long hairs. *Posterior Part*. Fig. 8, *a*, *h*. A short broad upstanding plate ending in a short point ; a short broad bristle attached near end on the outer side.

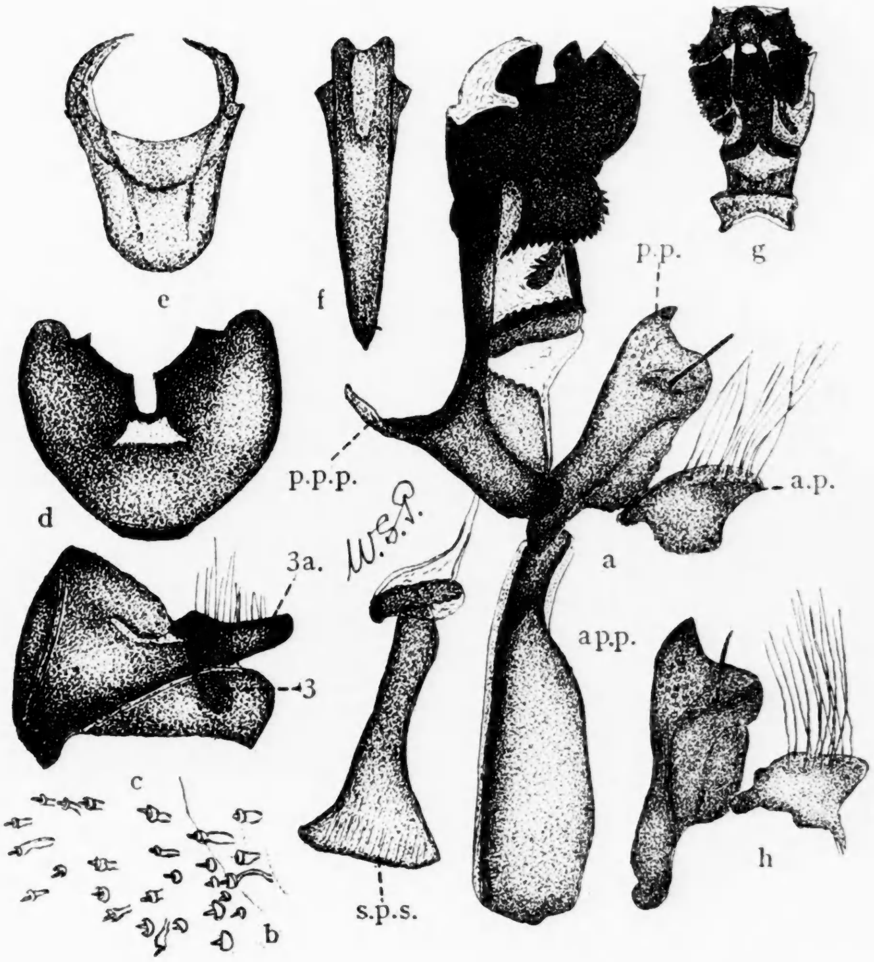

FIG. 8. *a*.—Phallosome, sperm pump sclerite, apodeme, posterior process of phallosome and one paramere of *bequaerti* in side view ; lettering as in fig. 3 ; *b*.—Some sensory spines from posterior part of paramere ; *c*.—Ninth coxite in side view showing posterior prolongation fused with tergum 10 ; *d*.—Fifth sternum ; *e*.—Ninth tergo-sternum ; *f*.—Ventral view of fused anal cerci ; *g*.—Dorsal view of phallosome ; *h*.—One paramere showing two parts.

SALIENT DIAGNOSTIC CHARACTERS OF FEMALE TERMINALIA. OVIPOSITOR. SEGMENTS 6, 7, 9 and 10. The fully extended ovipositor and the several sclerites are illustrated in fig. 9 ; note the long V-shaped portion of tergum 5 which fits into the incision of tergum 4. The differences in the shape, etc., of the sclerites of those of this species and of *luteola* can readily be made out by comparing figs. 5 ; 9. Tergum 6 is not completely divided into three parts. There is a marked difference in the structure of sternum 9 of *luteola* and *bequaerti*, for in the latter there is a small heart-shaped portion which is folded back and forms

the anterior wall of the genital opening (cf. fig. 5, *l*, with fig. 9, *g*). The signum of the uterus of *bequaerti* is larger and not so strongly chitinized, and the spermathecae (fig. 9, *k*) are much longer and narrower.

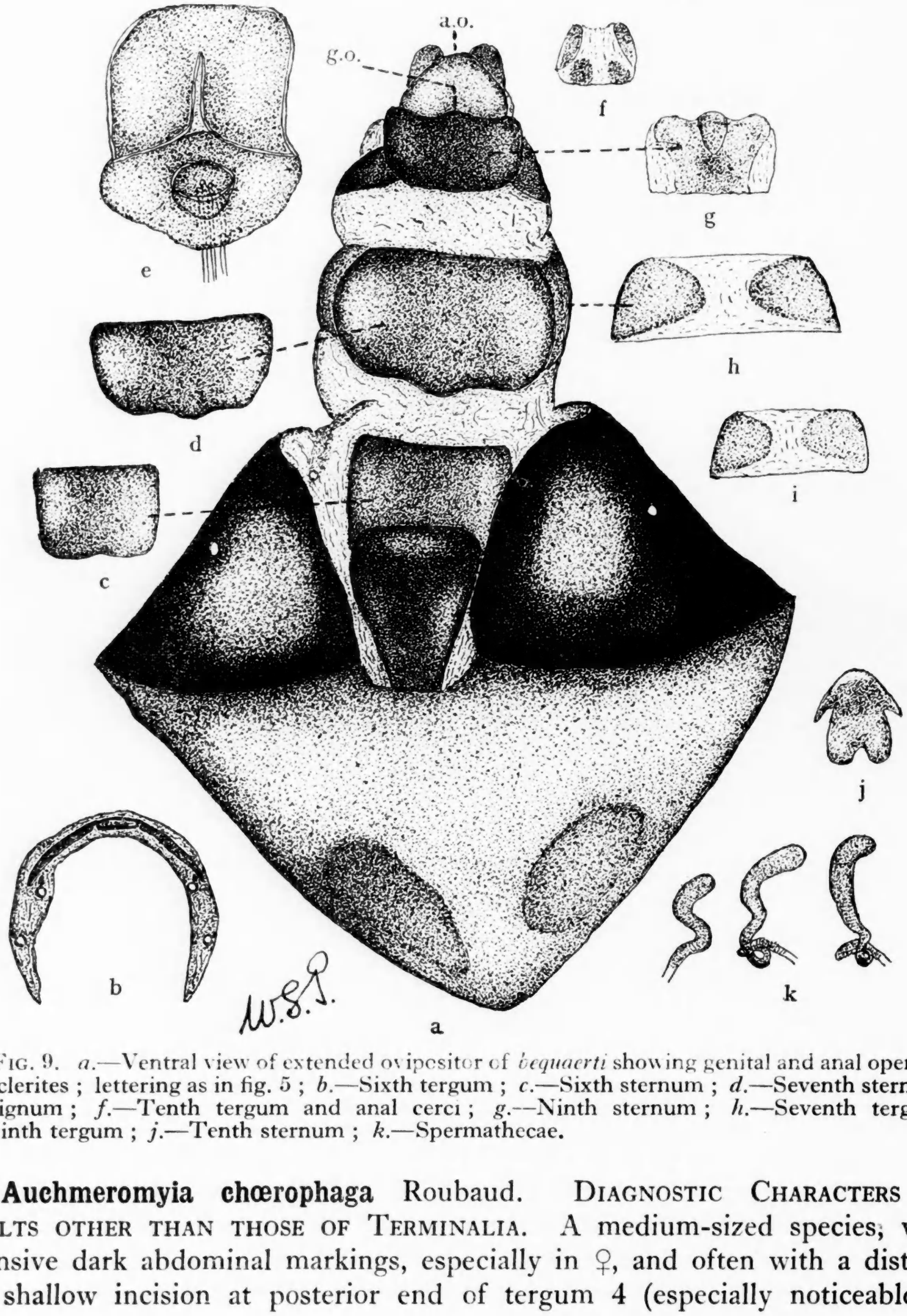

FIG. 9. *a.*—Ventral view of extended ovipositor of *bequaerti* showing genital and anal openings and sclerites ; lettering as in fig. 5 ; *b.*—Sixth tergum ; *c.*—Sixth sternum ; *d.*—Seventh sternum ; *e.*—Signum ; *f.*—Tenth tergum and anal cerci ; *g.*—Ninth sternum ; *h.*—Seventh tergum ; *i.*—Ninth tergum ; *j.*—Tenth sternum ; *k.*—Spermathecae.

Auchmeromyia chœrophaga Roubaud. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF TERMINALIA. A medium-sized species, with extensive dark abdominal markings, especially in ♀, and often with a distinct but shallow incision at posterior end of tergum 4 (especially noticeable in

specimens from West Africa, as noted by Roubaud). ♂. *Head.* Eyes widely separated ; vertex considerably less than half width of an eye. *Thorax.* Mesonotum with a pair of well-marked, broad, dark stripes reaching almost to scutellum. *Abdomen.* Fig. 6, *e.* Terga 1 and 2 yellow, sometimes with a very narrow black posterior band ; tergum 3 yellow, with a narrow dark posterior band and an indefinite dark median stripe ; tergum 4 black, except for anterior border which is yellow ; tergum 5 black, except for distal end which is yellow.

♀. *Head.* Eyes widely separated ; vertex about three-quarters width of an eye. *Thorax.* Mesonotal black stripes wide and well marked. *Abdomen.* Fig. 6, *f.* Terga 1 and 2 mainly black with some yellow patches ; tergum 3 black with some yellow admedian patches ; tergum 4 black, and in West African forms with a distinct posterior incision ; tergum 5 black, except end which is yellow.

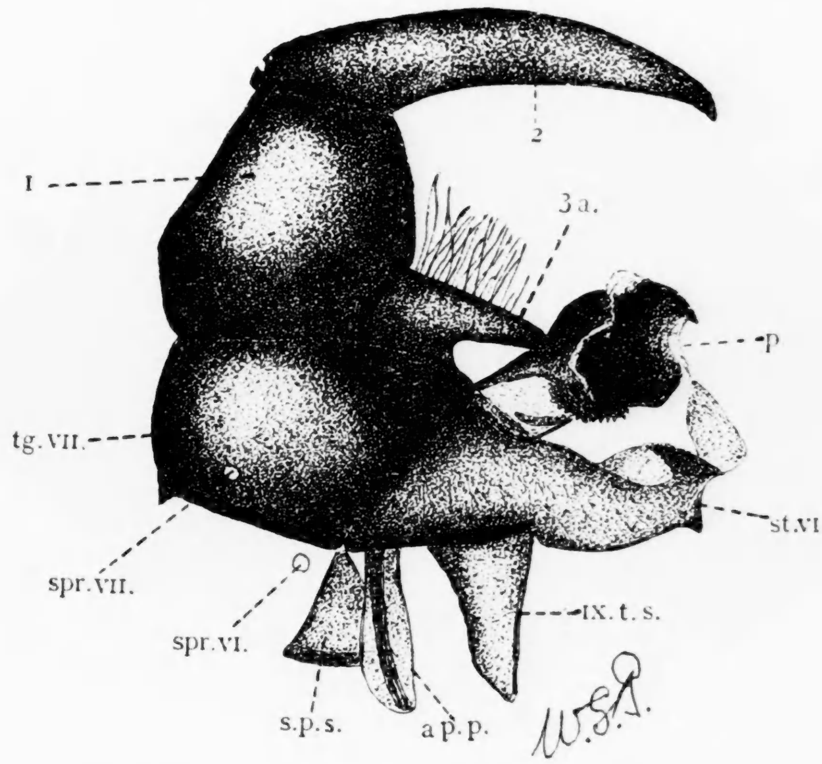

Fig. 10. Seventh and tenth terga, sixth sternum, anal cerci, ninth coxite and phallosome of *chœrophaga* in side view ; lettering as in fig. 2.

Salient Diagnostic Characters of Male Terminalia. The ♂ terminalia with the anal cerci drawn back are illustrated in side view in fig. 10, and the details of the phallosome, etc., in fig. 11. Sclerites. Sternum 5 as in fig. 11, *b*, which shows lateral lobes and smaller processes ; tergum 6 is wanting. Terga 7 and 10 and their relations to the ninth coxites and anal cerci are illustrated in fig. 10, and are very similar to those of the other species. Ninth tergo-sternum as in fig. 11, *d.*

APPENDAGES. *Ninth Coxites.* Lateral view. *Distal Segment.* Fig. 11, *c.* A short, rounded, finger-like process turned inwards at end and armed with numerous hairs ; posterior prolongation largely fused with tergum 10. *Proximal Segment.* Fig. 11, *c.* A rather wide plate fused with its fellow. *Anal Cerci.* Lateral view. Fig. 10. Completely fused, forming a rather long stout rod with broad blunt end ; ventral view as in fig. 11, *e.*

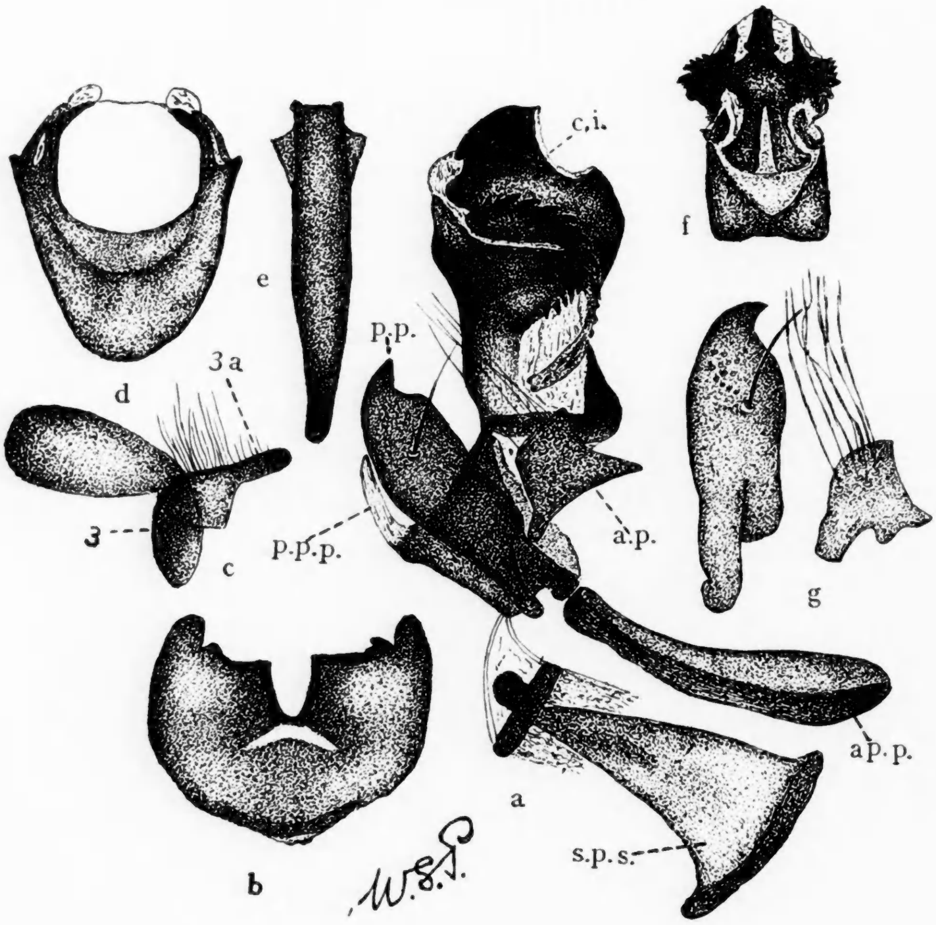

FIG. 11. *a.*—Phallosome, sperm pump sclerite, posterior process and one paramere of *chœrophaga* in side view ; *c.i.*—Crescentic incision of ejaculatory duct of phallosome ; other lettering as in fig. 3 ; *b.*—Fifth sternum ; *c.*—Ninth coxite showing posterior prolongation and two parts of coxite ; *d.*—Ninth tergo-sternum ; *e.*—Ventral view of fused anal cerci ; *f.*—Dorsal view of phallosome ; *g.*—Two parts of paramere.

Phallosome. Lateral view. Fig. 11, *a.* Short and heavily chitinized. Proximal portion short and bearing the narrow, rather longer, posterior process. Ventral part of distal portion wide and expanded into a serrated lateral flap ; dorsal part consisting of a large serrated flap on each side ; in addition there is a narrow lateral spined portion near the proximal end. Opening of ejaculatory duct seen as a characteristic crescentic incision in side view (fig. 11, *a*). *Posterior Process of Phallosome.* Fig. 11, *a.* As noted above. *Apodeme of Phallosome.* Fig. 11, *a.* Long and not very broad. *Sperm Pump Sclerite.* Fig. 11, *a.* Large, with a characteristic cap. *Parameres.* Lateral view. *Anterior Part* Fig. 11, *a, g.*

A small plate, similar to that of *luteola*, *boueti* and *bequaerti*, with about 6 long hairs. *Posterior Part.* Fig. 11, *a*, *g*. A long, upstanding, broad plate with a rather short stout bristle about middle, and the usual sensory spines.

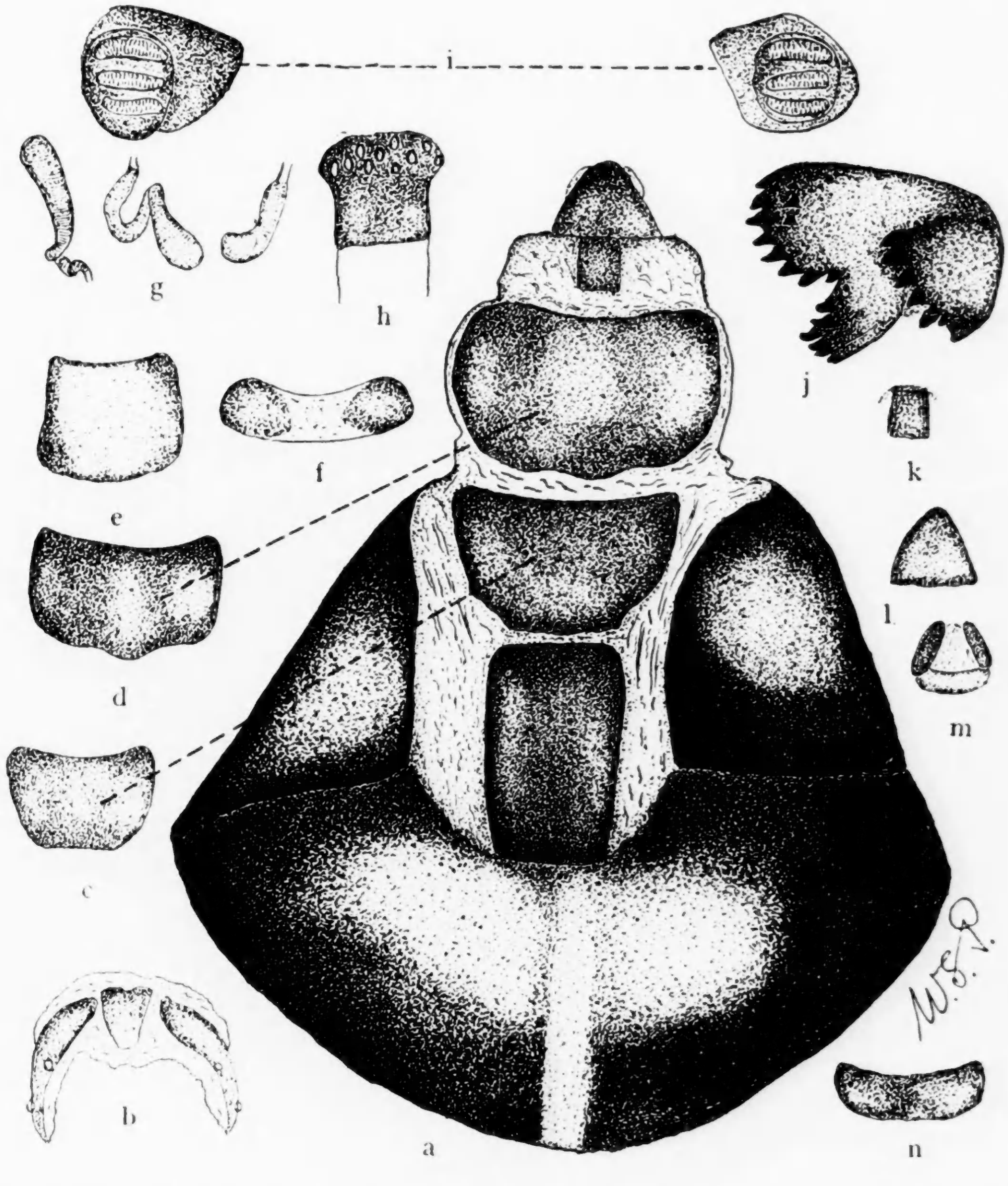

FIG. 12. *a.*—Ventral view of extended ovipositor of *chœrophaga* showing various sclerites ; *b.*—Sixth tergum ; *c.*—Sixth sternum ; *d.*—Seventh sternum ; *e.*—Signum ; *f.*—Seventh tergum ; *g.*—Spermathecae ; *h.*—Anterior spiracle of third larva ; *i.*—Posterior spiracles of same showing the small plates enclosing slits ; *j.*—Cutting plate of third larva ; *k.*—Ninth sternum ; *l.*—Tenth sternum ; *m.*—Tenth tergum and anal cerci ; *n.*—Ninth tergum.

SALIENT DIAGNOSTIC CHARACTERS OF FEMALE TERMINALIA. OVIPOSITOR. SEGMENTS 6, 7, 9 and 10. The fully extended ovipositor and the several sclerites forming it are illustrated in fig. 12 ; these drawings should be compared with those of the other species in order to note the differences in the sclerites, etc. Tergum 6 (fig. 12, *b*) is feebly developed and consists of three distinct plates.

Sternum 7 (fig. 11, *d*) is large, as in some of the other species, particularly *luteola*. The signum of the uterus (fig. 12, *e*) is relatively small and lightly chitinized, and two of the spermathecae (fig. 12, *g*) are very long. The cutting plate (fig. 12, *j*) of the larva is very similar to that of *luteola*, as are also the posterior spiracles ; the plates (fig. 12, *i*), however, are much more widely separated ; the anterior spiracle (fig. 12, *h*), too, is very like that of *luteola*.

Auchmeromyia boueti Roubaud. Diagnostic Characters of Adults other than those of Terminalia. Like *bequaerti* and *chœrophaga*, a medium-sized yellow species, the terga of abdomen not showing any marked inequality in length, and tergum 4 not incised.

♂. *Head.* Eyes widely separated ; vertex about one-third width of an eye. *Thorax.* Mesonotum with a pair of faintly marked admedian stripes only extending a short distance behind suture. *Abdomen.* Fig. 6, *c*. Terga 1 and 2 yellow ; tergum 3 yellow, with two dark spots or stripes on each side of middle line of posterior border ; tergum 4 yellow, with two rather better marked larger spots on each side of middle line of posterior border extending slightly along it ; tergum 5 yellow.

♀. *Head.* Eyes widely separated ; vertex equal to half width of an eye. *Thorax.* Mesonotum marked much as in ♂, dark stripes extending to end just in front of scutellum. *Abdomen.* Fig. 6, *d*. Terga 1 and 2 yellow, with (in some

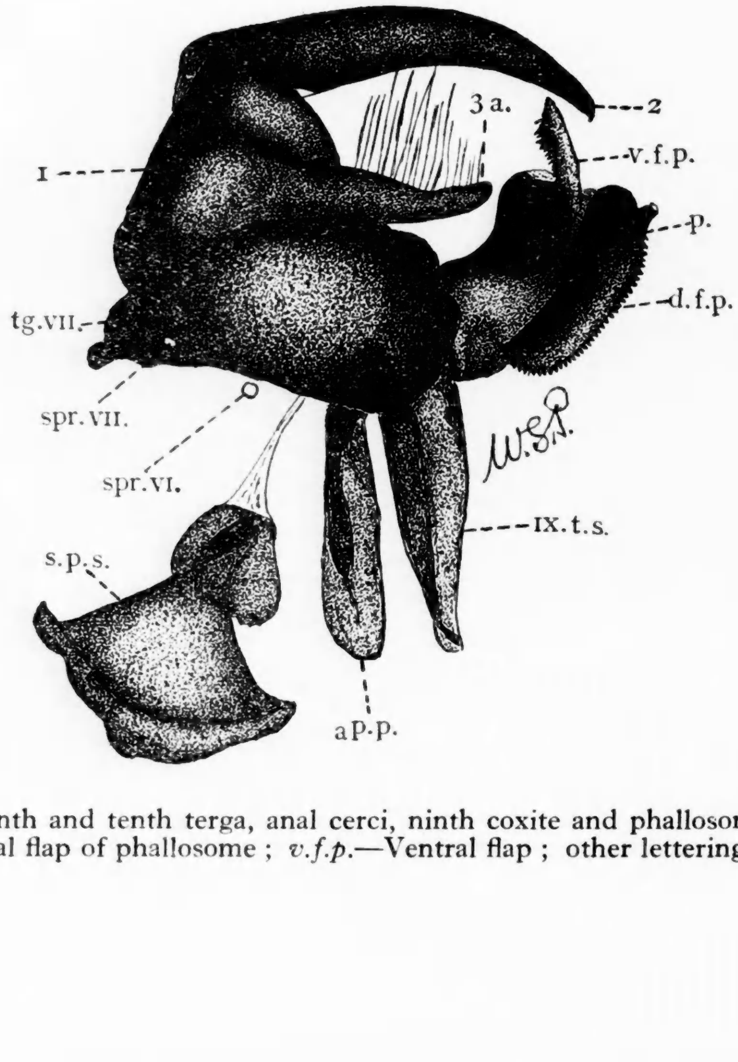

Fig. 13. Seventh and tenth terga, anal cerci, ninth coxite and phallosome of *boueti* in side view ; *d.f.p.*—Dorsal flap of phallosome ; *v.f.p.*—Ventral flap ; other lettering as in fig. 2.

specimens) a faint dark band at outer part of posterior border ; tergum 3 yellow with a median triangular-shaped dark stripe at posterior end, and a rather broad dark posterior band ; tergum 4 mostly black except for a median light-yellow stripe ; sometimes slightly incised ; tergum 5 yellow with a rather large round black spot on each side of middle line of anterior border.

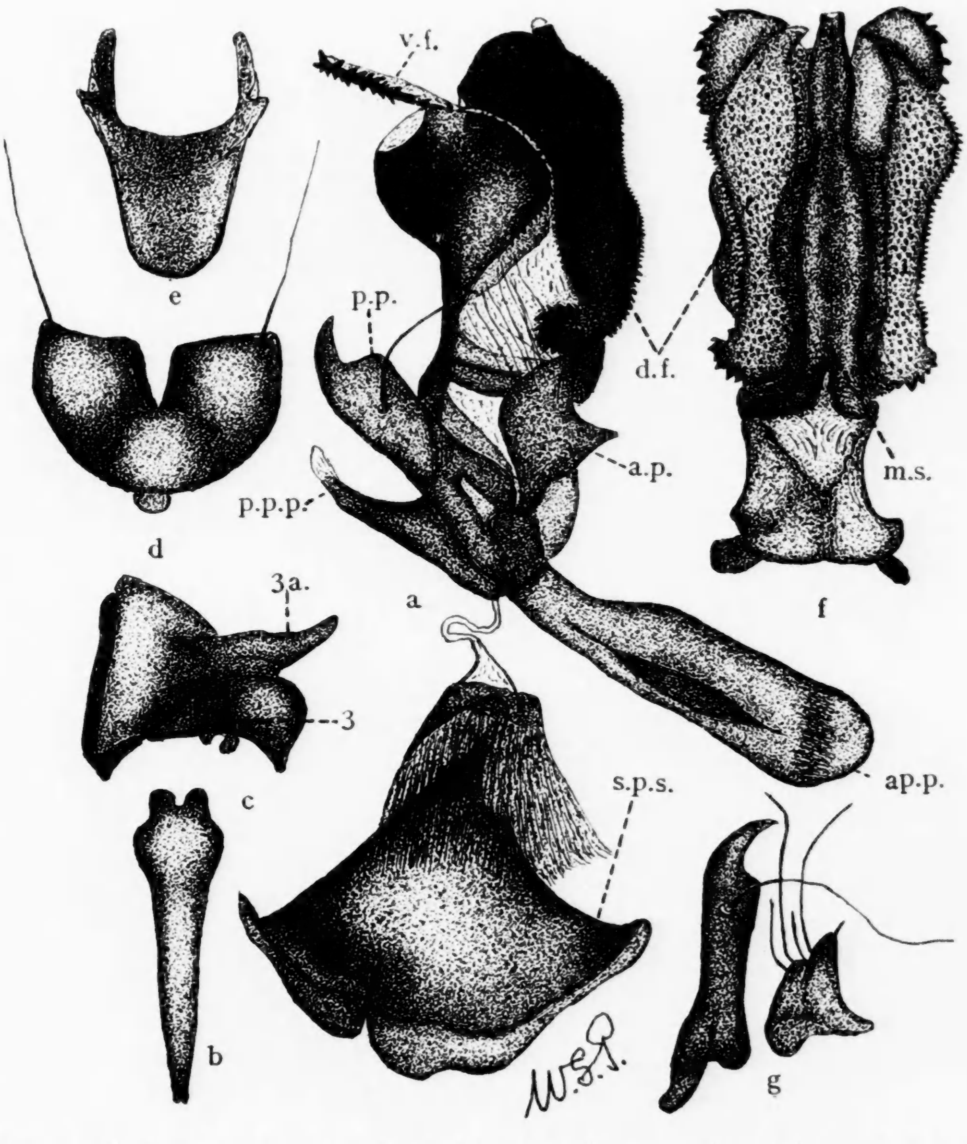

FIG. 14. *a.*—Phallosome showing flaps (dorsal, *d.f.* ; ventral, *v.p.*), sperm pump sclerite, apodeme, posterior process and one paramere of *boueti* in side view ; other lettering as in fig. 3 ; *b.*—Ventral view of fused anal cerci ; *c.*—Ninth coxite (proximal segment—3 ; distal segment—3*a*) and part of tergum 10 in side view ; *d.*—Fifth sternum ; *e.*—Ninth tergo-sternum ; *f.*—Dorsal view of phallosome showing flap and median shaft (*m.s.*) ; *g.*—One paramere showing two parts.

SALIENT DIAGNOSTIC CHARACTERS OF MALE TERMINALIA. The ♂ terminalia with anal cerci drawn back are illustrated in fig. 13, and the details of phallosome, etc., in fig. 14. SCLERITES. Sternum 5 as in fig. 14, *d* ; tergum 6 is wanting. Tergum 7 and 10 and their relations to each other, to the ninth coxites and to

the anal cerci are shown in fig. 13, and as they are very similar to those of *luteola* and *bequaerti* it is unnecessary to describe them. The ninth tergo-sternum as in fig. 14, *e*.

APPENDAGES. *Ninth Coxites*. Lateral view. *Distal Segment*. Figs. 13; 14, *c*. A rather long, flattened, finger-like process, turned in at the end, with many long hairs. *Proximal Segment*. Fig. 14, *c*. A short stout rod joined to its fellow. *Anal Cerci*. Lateral view. Fig. 13. Fused, forming a rounded curved rod, similar to that of *bequaerti*, and slightly bifid at end; ventral view as in fig. 14, *b*.

Phallosome. Lateral view. Fig. 14, *a*. Short, broad and heavily chitinized (longer and more massive than that of *bequaerti*). Proximal portion as in other species. Distal portion divided into two parts, the ventral consisting of the struts expanding distally into rounded, serrated plates, and a long strongly serrated ventrally directed flap (fig. 14, *a*); dorsal part consisting of the median rounded shaft and a long, very characteristic, serrated flap on each side; its structure is well shown in figs. 13; 14, *a*; the two portions are united as in the other species. *Posterior Process of Phallosome*. As in fig. 14, *a*. *Apodeme of Phallosome*. Fig. 14, *a*. Long and broad in side view. *Sperm Pump Sclerite*. Fig. 14, *a*. A very large fan-shaped plate with a chitinous cap. *Parameres*. Lateral view. *Anterior Part*. Fig. 14, *a*, *g*. Similar to that of *luteola* and *bequaerti* but with fewer hairs. *Posterior Part*. Fig. 14, *a*, *g*. A long, rather narrow, upstanding plate, with a long pointed end and a very long hair on the outer side near the end.

SALIENT DIAGNOSTIC CHARACTERS OF FEMALE TERMINALIA. OVIPOSITOR. SEGMENTS 6, 7, 9 and 10. The fully extended ovipositor and the several sclerites are illustrated in fig. 15. For the differences in the shape of those of *luteola*, *bequaerti*, *chœrophaga* and *boueti*, compare figs. 5; 9; 12; 15. Tergum 6 is as in *bequaerti*. There is a marked difference in the shape of sternum 9 in the four species, and, as in *bequaerti*, there is a small, rounded, chitinous portion turned over, forming the anterior wall of the genital opening. The signum of the uterus (fig. 15, *j*) is very large and of a characteristic shape; two of the spermathecae (fig. 15, *g*) are very long, narrow and convoluted.

Auchmeromyia prægrandis Austen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF TERMINALIA. A very large yellow species with normal abdominal terga and squama with numerous silky hairs on upper surface. ♂. *Head*. Eyes widely separated; vertex about equal to the width of an eye. *Thorax*. Mesonotum with two broad black stripes not reaching posterior border. *Abdomen*. Fig. 16. Terga 1, 2 and 3 yellow; tergum 4 yellow with a broad posterior black band (usually half length of segment); tergum 5 black, except for a slight median yellow stripe, and armed with a row of strong bristles along posterior border. Usually the hairy yellow terga 7 and 10 are visible.

♀. *Head*. Eyes widely separated; vertex about equal to the width of an eye. *Thorax*. Mesonotum as in ♂. *Abdomen*. Fig. 16. Terga 1 and 2 as in

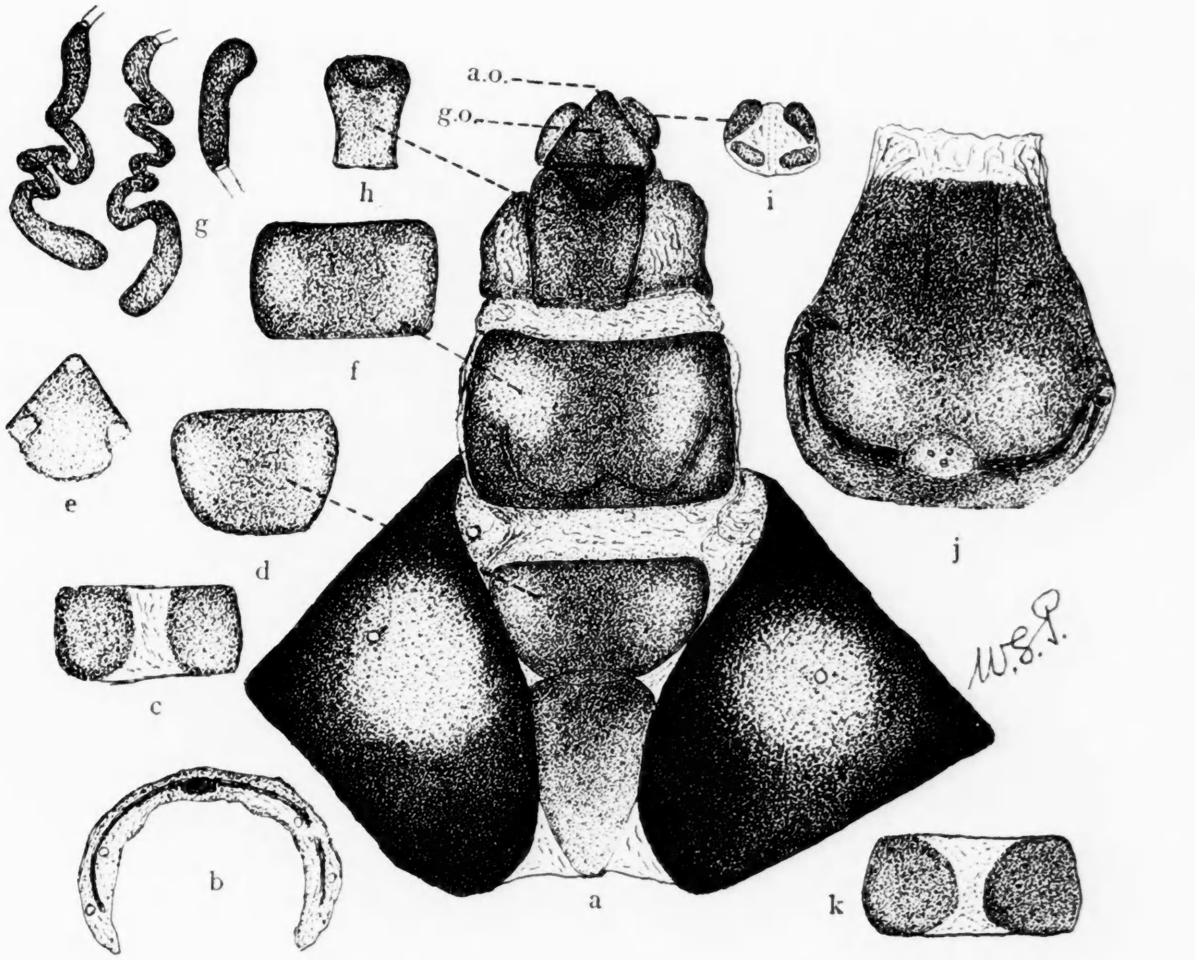

FIG. 15. *a.*—Ventral view of extended ovipositor of *boueti* showing genital and anal openings and sclerites; *b.*—Sixth tergum; *c.*—Ninth tergum; *d.*—Sixth sternum; *e.*—Tenth sternum; *f.*—Seventh sternum; *g.*—Spermathecae; *h.*—Ninth sternum; *i.*—Tenth tergum and anal cerci; *j.*—Signum; *k.*—Seventh tergum.

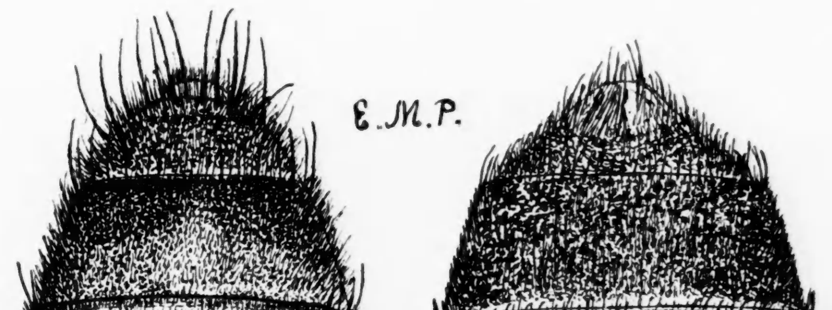

FIG. 16. ♂ and ♀ abdomen of *prægrandis*.

♂ ; tergum 3 yellow with a well-marked narrow black posterior band expanding laterally ; tergum 4 black, except for a narrow anterior yellow band ; tergum 5 mainly yellow with antero-lateral dark areas, the straight narrow extremity armed with a row of strong bristles. Ventral edges of tergum 5 armed with a row of very stout bristles, as in fig. 20.

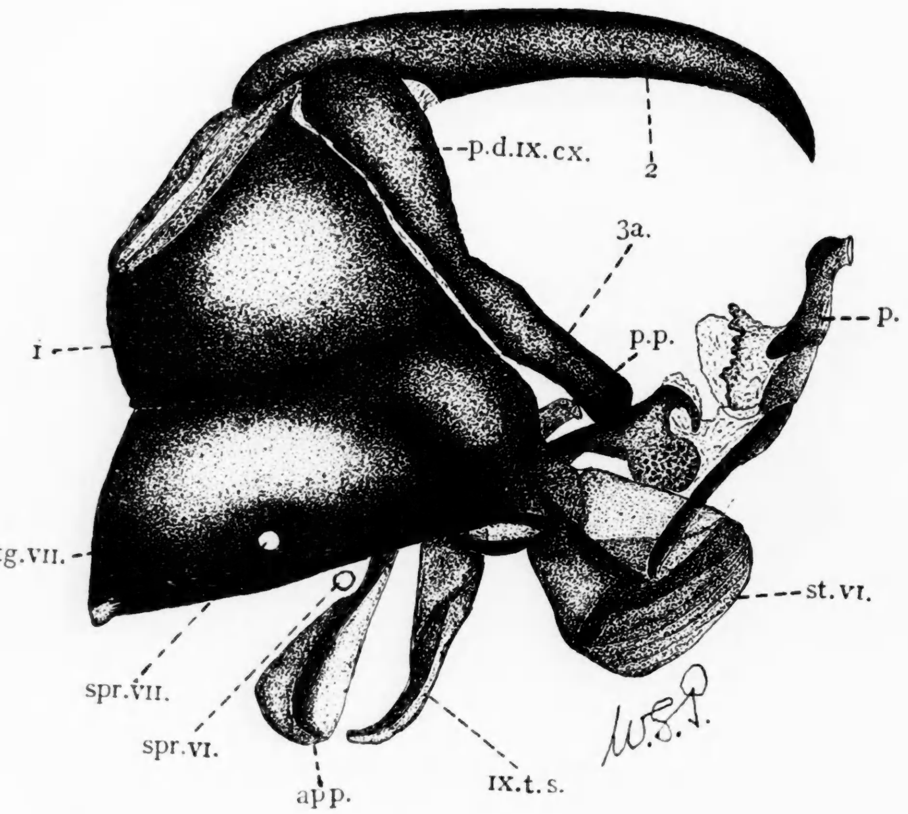

Fig. 17. Seventh and tenth terga, sixth sternum, anal cerci, ninth coxite and phallosome of *prægrandis* in side view ; *p.*—Phallosome ; *p.p.*—Posterior paramere ; other lettering as in fig. 2.

Salient Diagnostic Characters of Male Terminalia. The ♂ terminalia with anal cerci drawn back are illustrated in fig. 17 (smaller scale than those of four other species) ; the details of the phallosome, etc., are illustrated in fig. 18. Sclerites. Sternum 5 as in fig. 18, *c*. Tergum 6 narrow, extending laterally, spiracle 6 located on a detached pear-shaped plate (not illustrated in fig. 17). Terga 7 and 10 and their relations to the ninth coxites and anal cerci are illustrated in fig. 17 and therefore need not be noted. Ninth tergo-sternum a massive plate, as shown in fig. 18, *e*.

Appendages. *Ninth Coxite.* Lateral view. *Distal Segment.* Figs. 17 ; 18, *b*. A long broad plate with square end turned in, with numerous long hairs, and with a long, rather narrow, posterior extension. *Proximal Segment.* Fig. 18, *b*. A short, stout, rod-like plate fused with its fellow. *Anal Cerci.* Lateral view. Fig. 17. Fused, forming a long curved rounded rod fitting into notch of sternum 5 (fig. 18, *c*) ; ventral view as in fig. 18, *f*.

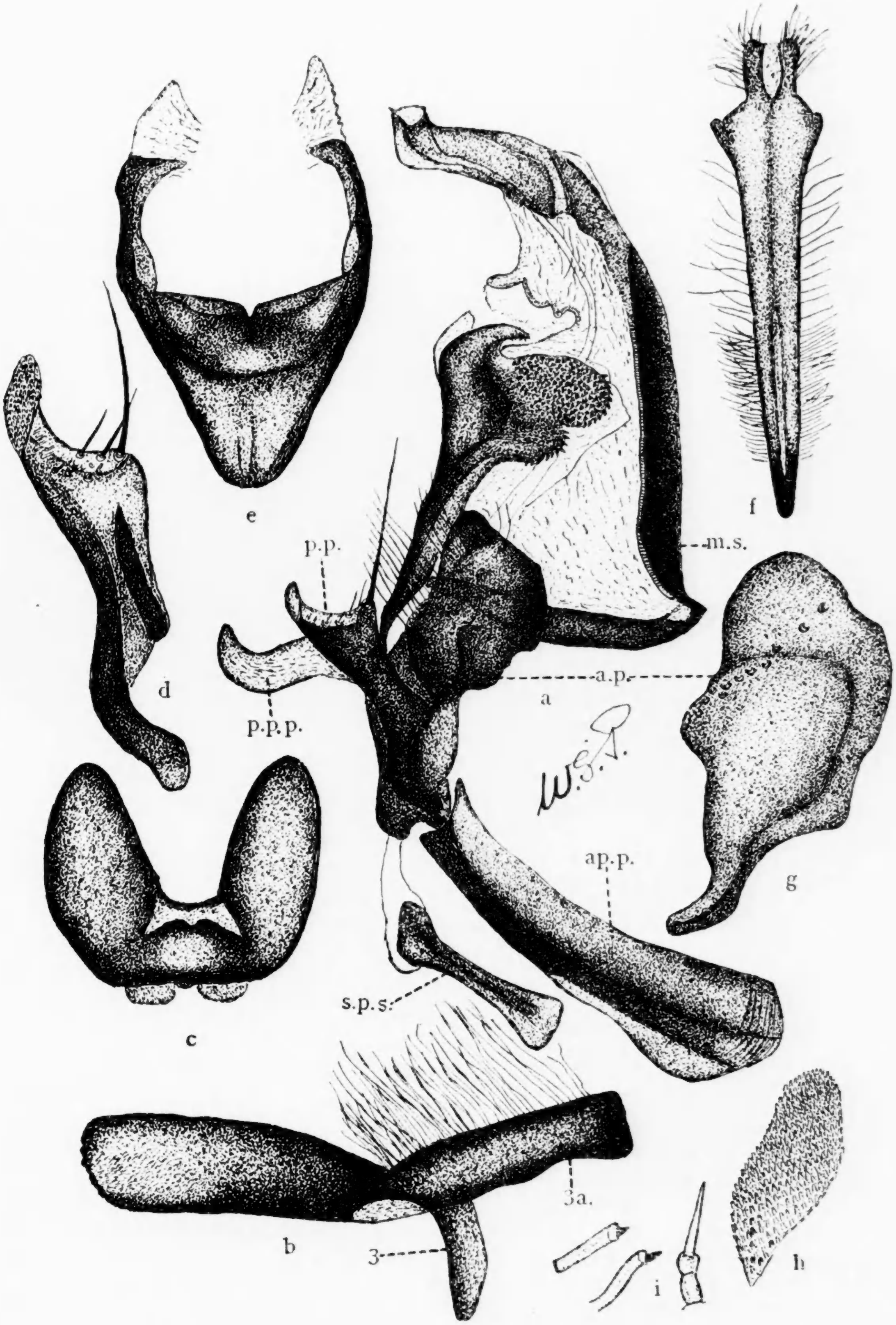

FIG. 18. *a.*—Phallosome, sperm pump sclerite, apodeme, posterior process and one paramere of ***prægrandis*** ; lettering as in fig. 3 ; *b.*—Ninth coxite showing two parts and posterior prolongation of distal segment ; *c.*—Fifth sternum ; *d.*—Posterior part of right paramere ; *e.*—Ninth tergo-sternum ; *f.*—Ventral view of fused anal cerci ; *g.*—Anterior paramere of same ; *h.*—Flat spines at end of posterior paramere ; *i.*—Three longer sensory spines on outer part of posterior paramere.

Phallosome. Lateral view. Figs. 17 ; 18, *a*. Proximal portion long and narrow, bearing the short, narrow, bent, posterior process. Struts forming ventral part of distal portion narrow, expanding into a strongly serrated flap ; median shaft of distal portion elongated, the distal part expanded and of lighter chitin, bent ventrally, and carrying the opening of ejaculatory duct at end, as illustrated in fig. 18, *a*. *Posterior Process of Phallosome*. Fig. 18, *a*. As already noted. *Apodeme of Phallosome*. Fig. 18, *a*. Long and broad. *Sperm Pump Sclerite*. Fig. 18, *a*. Long and narrow. *Parameres*. Lateral view. *Anterior Part*. Fig. 18, *a*, *g*. A stout broad plate with a row of 12–13 long hairs on outer surface. *Posterior Part*. Fig. 18, *a*, *d*. A stout upstanding plate ending in a blunt point with many appressed spines, and a broad shelf on which are located 1 long and 2–4 shorter bristles.

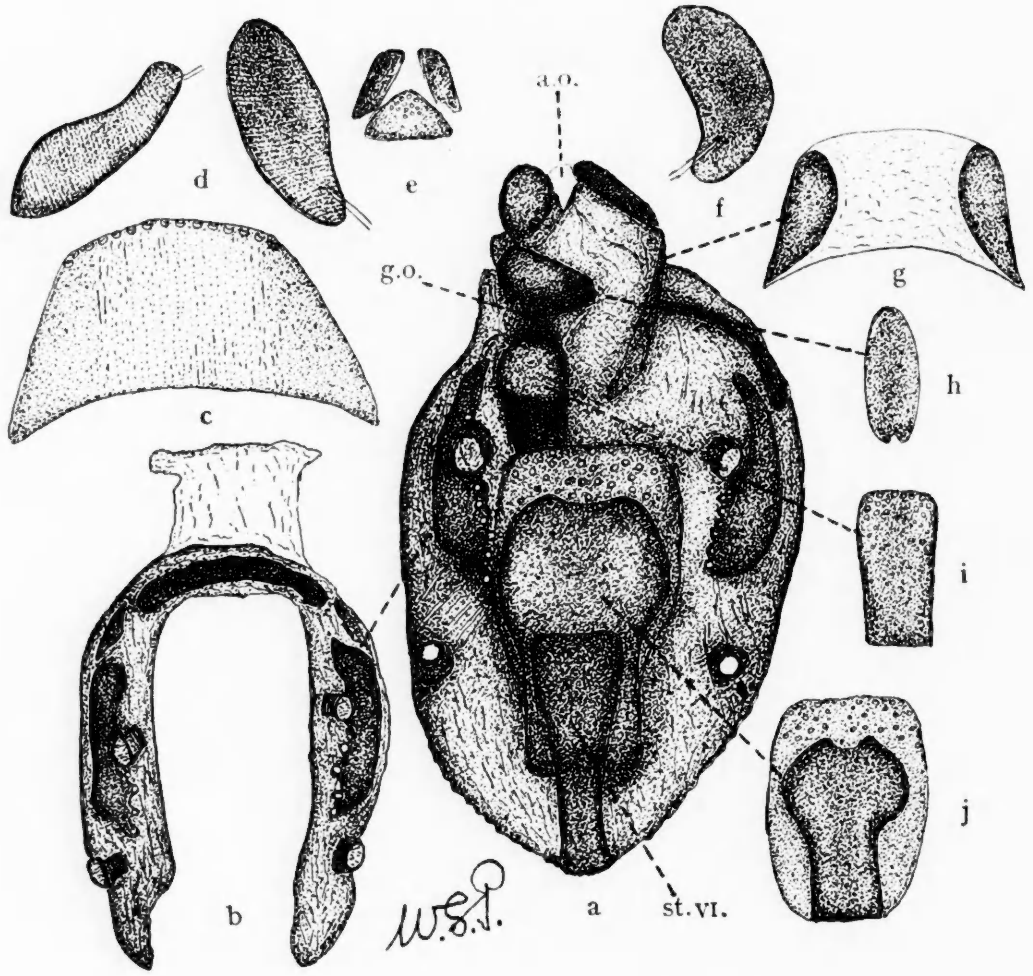

FIG. 19. *a*.—Ventral view of extended ovipositor of *prægrandis* showing genital and anal openings and sclerites ; *b*.—Sixth tergum ; *c*.—Seventh tergum ; *d*., *f*.—Spermathecae ; *e*.—Tenth tergum and anal cerci ; *g*.—Ninth tergum ; *h*.—Tenth sternum ; *i*.—Ninth sternum ; *j*.—Seventh sternum.

SALIENT DIAGNOSTIC CHARACTERS OF FEMALE TERMINALIA. OVIPOSITOR. SEGMENTS 6, 7, 9 and 10. The fully extended ovipositor and the several sclerites are illustrated in fig. 19. The ventral view of end of abdomen showing how

tergum 5 closes the genital sulcus is illustrated in fig. 20. The ovipositor is very short. Tergum 6 consists of three plates, as shown in fig. 19, *b*. The remaining sclerites, though structurally similar to those of the other four species, are narrowed, elongated and even reduced, owing to the closing of the genital sulcus by the ventral edges of tergum 5. The chitinous plate of the uterus is wanting. The spermathecae are pear-shaped, and the duct arises from the side, not from the end.

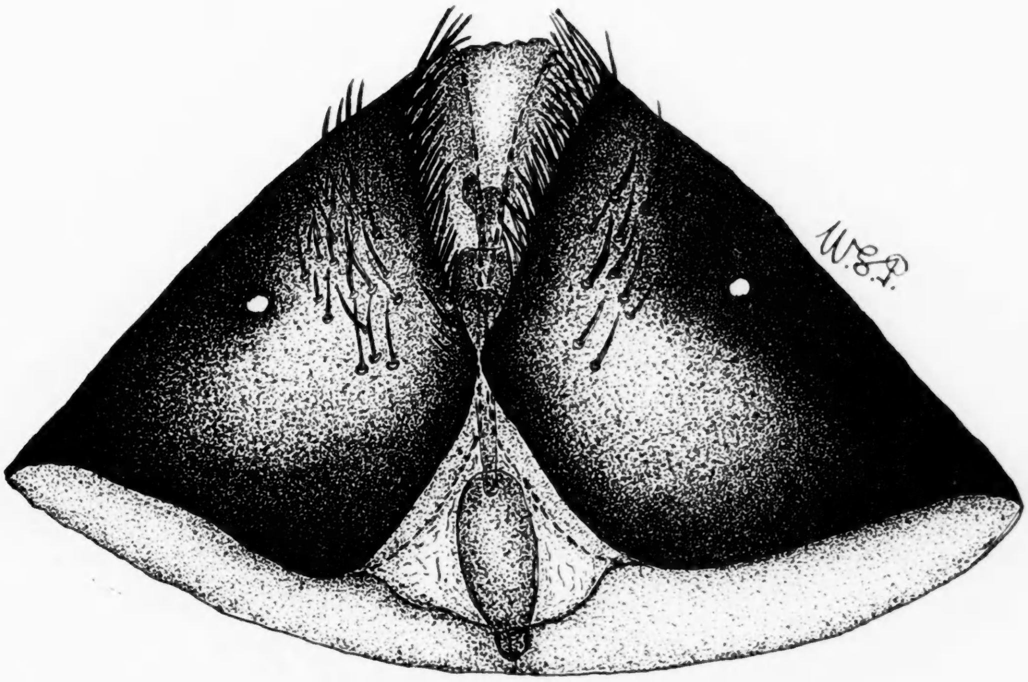

FIG. 20. Ventral view of end of abdomen of ♀ *prægrandis* showing the row of strong bristles along ventral edges of tergum 5 ; the dotted lines indicate the normal position of the edges.

KEY TO THE ♂♂ OF THE SPECIES OF *Auchmeromyia*

1. A very large species (14–17 mm.) ; squama with silky hairs on upper surface ; dorso-median shaft of phallosome with a characteristic long prolongation bent ventrally at end (figs. 17 ; 18, *a*) ... ... *prægrandis*.
   Much smaller species (9–13·5 mm.) ; squama without hairs on upper surface ; phallosome short and massive 2
2. Species with tergum 3 (apparent 2nd) about 1½ times longer than tergum 4 (fig. 1) ; anal cerci a long, round, curved rod bifid at end (fig. 3, *a*) ; phallosome a short massive structure, as illustrated in fig. 3, *a* ... *luteola*.
   Species in which tergum 3 is of normal length 3
3. Mesonotal black stripes not well marked ; abdomen mainly yellow with few markings (fig. 6, *c*) ; anal cerci of moderate length and slightly bifid at end (fig. 13) ; sternum 5 without any lateral lobes (fig. 14, *d*) ; phallosome with a long serrated flap on ventral part, projecting ventrally (fig. 14, *a*) ; a long chitinous flap on each side of median shaft (fig. 14, *a*) ... ... *boueti*.
   Mesonotal black stripes well marked and broad ; abdomen better marked, especially terga 4 and 5 (fig. 6) ; anal cerci not bifid at end ; sternum 5 with well-marked lateral lobes and a smaller projection on the inner side of each 4

4. Posterior border of tergum 4 straight, markings as in fig. 6, *e* ; anal cerci rather long, stout and broadly rounded at end (fig. 10) ; dorsal portion of phallosome with a large strongly serrated flap on each side, ventral portion (strut) ending in a rather narrow serrated expansion (fig. 11, *a*) ; opening ejaculatory duct seen as a characteristic crescentic incision in side view (fig. 11, *a*) ... ... *chœrophaga.*
   Posterior border of tergum 4 with a slight but distinct incision, and markings as in fig. 6, *b* ; anal cerci shorter and ending in characteristic beak-like hook when seen in side view (fig. 7) ; dorsal portion of phallosome without a large lateral flap ; ventral portion (strut) ending in a large lateral serrated expansion (fig. 8, *a*) ; ejaculatory duct opening not crescentic-shaped (fig. 8, *a*) ... ... *bequaerti.*

Key to the ♀♀ of the Species of *Auchmeromyia*

1. A very large species (14–16 mm.) ; squama with silky hairs on upper surface ; ventral edges of tergum 5 armed with strong bristles and almost meeting, thus enclosing terminalia (fig. 20) ... ... *prægrandis.*
   Much smaller species (9–13·5 mm.) ; squama bare 2
2. Species with tergum 3 (apparent 2nd) about twice the length of tergum 4 (fig. 1) ; tergum 5 triangular-shaped, turned up at end, and ventral edges almost meeting, thus enclosing terminalia (fig. 4) ... ... *luteola.*
   Species with tergum 3 of normal length 3
3. Species with tergum 4 markedly incised on posterior border and markings as in fig. 6, *a* ... ... *bequaerti.*
   Species with tergum 4 not incised as in *bequaerti* (sometimes slightly so, as *chœrophaga* from West Africa) 4
4. Terga 3, 4 and 5 black or nearly so ; tergum 4 sometimes slightly incised *chœrophaga.*
   Terga 3, 4 and 5 mainly yellow with black markings, as shown in fig. 6, *d* *boueti.*

There should be no difficulty in recognizing the ♂ and ♀ *prægrandis* ; its size, the presence of hairs on upper surface of squama, the characteristic phallosome and the strong bristles on the ventral edges of tergum 5 in ♀ all render identification easy. Similarly, the ♂ and ♀ *luteola* can be readily identified by the long 3rd tergum in both sexes. The ♀ *bequaerti* can also readily be recognized by the deep incision on the posterior border of tergum 4 ; the ♂ *bequaerti* can be recognized by the beak-like hook at the end of the anal cerci, the characteristic phallosome (fig. 7), and the slight incision at the posterior border of tergum 4. The ♂ *chœrophaga* can be recognized by the fairly long blunt anal cerci, the characteristic curved opening of the ejaculatory duct (fig. 11, *a*), and the abdominal markings (fig. 6, *e*) ; the black abdomen of the ♀ is also characteristic. The ♂ *boueti* is a pale species, the markings on the abdomen being much reduced (fig. 6, *c*, *d*) ; the phallosome is very characteristic, with its long serrated dorsal flap (fig. 14, *a*). The markings of the ♀ *boueti* are fairly characteristic (fig. 6, *d*).

It is necessary to point out that the beginner may confuse species of *Auchmeromyia* (other than *prægrandis* and *luteola*) with species of *Bengalia*, and especially with *Cordylobia anthropophaga* ; the following notes may, therefore, be of some help in this direction. The species of *Bengalia* are large yellow muscids and are common in Africa. All have a short, to very short, conical proboscis, the labium is heavily chitinized and the prestomal teeth are well

developed ; they have, on the other hand, many characters in common with *Auchmeromyia*. In *Auchmeromyia* the proboscis is of normal length and structure, tapering to the end, the labium is not strongly chitinized and the prestonal teeth are small, for unlike the species of *Bengalia* none of them are predaceous. The ♂ *Cordylobia anthropophaga* is holoptic, the eyes practically meeting, whereas all the ♂♂ of *Auchmeromyia* are dichoptic, with the eyes well separated ; the terminalia too are very distinct. The ♀ *C. anthropophaga* is, however, not unlike some of the species of *Auchmeromyia*, and may be confused with them. In *C. anthropophaga* the outer vertical hairs (between the margins of the orbits and the inner vertical bristles) are black, well developed and arranged in two rows extending down well beyond the level of the antennae. In the ♀♀ of *Auchmeromyia* the outer vertical hairs are practically wanting, and the few there are are yellow. The last visible abdominal tergum (5th) in *anthropophaga* is broadly rounded, whereas in *Auchmeromyia* it is bluntly pointed.

GEOGRAPHICAL DISTRIBUTION OF THE SPECIES OF AUCHMEROMYIA. *A. luteola* is widely distributed in Africa, from Senegal in the west to the Sudan in the east, and as far south as the Cape. It is strictly associated with man. *A. boueti* is only known from West Africa. *A. chœrophaga* is also mainly a West African species. *A. bequaerti* is at present only known from East Africa. *A. prægrandis* is widely distributed in both West and East Africa, extending down to Natal. The four last species are associated with the Aard Vark (*Orycteropus* species) and the wart-hog (*Phacocherus*).

SYSTEMATIC POSITION OF THE GENUS AUCHMEROMYIA AND THE RELATIONSHIPS OF THE SPECIES. Before considering the systematic position of the genus, I should like to take this opportunity of making some points clear regarding these studies on the higher Diptera. In the first place, I am only too conscious of the fact that errors of interpretation are bound to creep into the work ; this is inevitable for at least two reasons. (1) Owing to the rapidity with which the work has to be done, the time at my disposal for completing it being very short, it is impossible to examine many species of genera, etc., which might throw additional and unexpected light, not only on the interpretation of the parts, but on the affinities and relationships of species ; moreover, in many instances I do not possess the necessary material. Were I to wait until I could examine many more species, the major part of my task would remain unaccomplished. I will without hesitation correct any errors when they are discovered. (2) It is impossible at present to be certain of the true homologies of some of the parts of the terminalia. I am also equally conscious of the fact that there are those who entirely disagree with my interpretations of the homologies of the parts of the terminalia. This too is inevitable. As no finality can be reached until the development of the parts has been traced through the larva and pupa on to the adult, there is no proof either that I am wrong or the reverse, and so I shall not deviate from the interpretations that I have adopted, the reasons for which I have explained in earlier papers. But, though there may be very diver-

gent views as to the homologies of the segments and appendages at the end of the abdomen of the ♂ and ♀ higher Dipteron, there are three structures which (call them what one likes—for this is of very minor importance) afford the only reliable clues to the relationships and affinities of species, genera, etc., provided that one makes certain that one is dealing with the same part in all. These are :—1. *Ninth Coxites* (paralobes, inferior claspers, editum, VII sternite, valvulae externae, etc.) ; 2. *Anal Cerci* (superior claspers, superior forceps, mesolobes, obere zange, VIII sternite, mesocerci, laminae genitale, etc.) ; 3. *Phallosome* (penis, either entire or in part) and *Parameres*. I attach most importance to the ninth coxites (appendages of the ninth segment). In the Diptera they consist normally of two segments—proximal and distal (also given many other names). The proximal, in the higher Diptera, always articulates with the sides of what I have called the ninth tergo-sternum (the plate which carries the phallosome and parameres), and is a lever ; the distal segments are normally lateral claspers, and I believe that this is the ancestral type ; it is well seen in such genera as *Hypoderma*, *Gasterophilus*, *Auchmeromyia*, *Bengalia*, *Syrphinae*, etc. The distal segment has become modified in several ways in relation to the changes in the structure of the ♀ terminalia. In the recent forms of the higher Diptera two striking changes have taken place. In the tribe Muscini, subfamily Muscinae, the distal segment has become reduced, and now the proximal segment forms the lateral clasper, as in *Musca*, *Stomoxys*, etc. In the tribe Anthomyini and in the Calliphorinae, the distal segments have migrated backwards and have come to lie close up against the outer sides of the anal cerci, and with them form dorso-ventral claspers of the end of the abdomen. In *Sarcophaga* the proximal segment has disappeared, and the distal segment is reduced to a triangular plate which takes no part in clasping, that now being entirely performed by the anal cerci, which appear in this genus (in some cases) to be inserted into the genital opening. In *Glossina* the proximal segment has also disappeared, and the distal is represented by a hairy pad (editum) on the antero-lateral edge of tergum 10.

The anal cerci of the ♂ in the primitive higher Diptera always appear to be small structures, as in *Hypoderma*, *Gasterophilus*, *Syrphinae*, etc., and this form is, I believe, nearest to the ancestral type. The anal cerci have also become modified in various ways in relation to the changes in the structure of the ♀ terminalia ; I hope to discuss these on another occasion. The phallosome and parameres afford important clues as to the relationships of species within a genus, as is brought out in this paper. The study of these parts in *Auchmeromyia*, and the process of copulation in *luteola*, has enabled me to reach certain conclusions as to the systematic position of the genus and the relationships and affinities of the species.

The position of the distal segment of the ninth coxite in *Auchmeromyia* has led to the conclusion that the segment is essentially a lateral clasper, and that in the ancestor that was its function. The structure of the ovipositor supports

this conclusion. But the change which has taken place in the method of oviposition, the fifth tergum being used as a plough, has led to the distal segments migrating forwards, until now they lie near the base of the phallosome and are used to evert the ventral edges of tergum 5 during copulation ; this is clearly seen in pairs of *luteola* taken *in cop.* Professor Roubaud describes the method of oviposition of *luteola* and *chœrophaga* as follows : ' In nature I have many times had the opportunity of observing the egg-laying, in quiet huts, dark and not much inhabited. The ♀ runs on the ground in all directions, trailing the extremity of the abdomen in the form of a ploughshare, tracing thus a furrow in the sand or dust. She seeks, with the point of her keel-like abdomen, crevices, holes or cracks in the ground filled with light soil. When a favourable substratum is found, that is to say sufficiently fine and soft, she sinks her abdomen deeply and deposits in this pulverent shelter an egg, rarely several ; she then moves further and recommences the same manoeuvre to scatter her eggs on the ground of the hut.' And again : ' The *Chœromyia* deposit their eggs in the humid earth at the bottom of the burrows of the Phacocheres and Orycteropes, either alone or in small groups. I have only observed the oviposition in captivity, and then only for *Ch. chœrophaga*, which is similar to that of *A. luteola*.' These interesting observations strongly support my conclusions, for in order to make a furrow it is necessary for the ventral edges of tergum 5 to be closely approximated, in which position they would impede copulation and thus make it necessary for the distal segments of the ninth coxites of the ♂ to evert the edges. In the case of *prægrandis*, which presumably oviposits in the same way, the ventral edges of tergum 5 have in addition a strong brush of bristles, and completely overlap the genital opening which lies in a deep sulcus (fig. 20). Here, then, we have the explanation of a striking modification in the structure and function of the distal segments of the ninth coxites in order to facilitate copulation, owing to the change in the structure of the end of the abdomen of the ♀, due to the adoption of a peculiar method of oviposition in a special habitat. Correlated with these changes, the anal cerci of the ♂ have become profoundly altered ; they have become elongated and fused, and now are used to grasp the end of the ♀ abdomen dorso-ventrally. The phallosome and parameres are structurally similar in the four species, *luteola*, *chœrophaga*, *bequaerti* and *boueti* (see illustrations), clearly demonstrating that they are closely related. The phallosome of *prægrandis*, on the other hand, seems at first sight to be so distinct from that of the others as to justify its being placed in a separate genus (*Chœromyia* Roubaud or *Parachœromyia* Villeneuve). A careful study of the phallosome of *boueti*, however, in my opinion clearly suggests that the two are related. I have tried to bring this out in fig. 21, in which the phallosome of *boueti* and of *prægrandis* (smaller scale) in side and dorso-ventral views are illustrated. If we remove the flap from the phallosome of *boueti* (fig. 21, *d*) it calls for no very great stretch of imagination to see that it is structurally similar to that of *prægrandis* ; the ventral flaps in each are not markedly different, for, if we reduce

that of *boueti*, we have that of *prægrandis*, and if we elongate the median shaft of the former the similarity is complete. It is interesting here to quote some remarks of Roubaud on the host relationships of these flies. He says : ' The parasitical specificity of *Chœromyia* must spread simply at the expense of animals

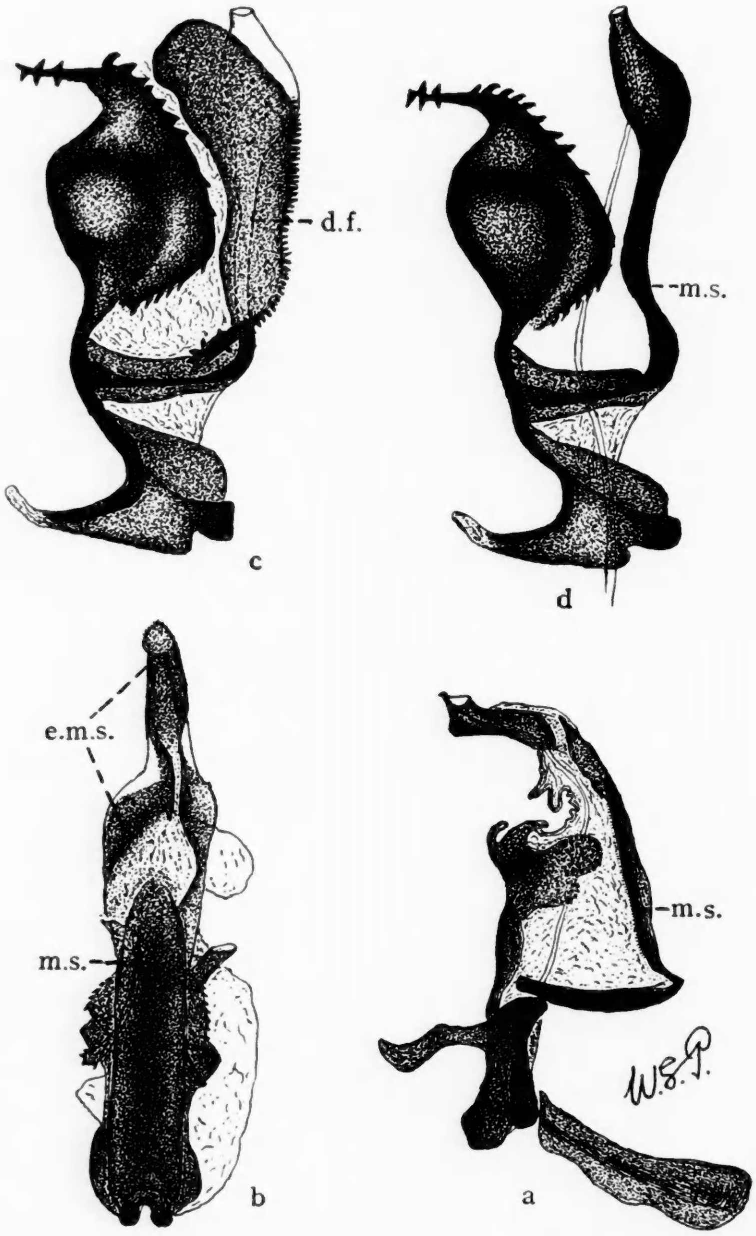

FIG. 21. *a.*—Lateral view of phallosome and apodeme of *prægrandis* ; *m.s.*—Median shaft ; *b.*—Dorsal view of phallosome of same ; *e.m.s.*—Elongation of median shaft ; *c.*—Lateral view of phallosome of *boueti* showing dorsal flap, *d.f.* ; *d.*—The same with dorsal flap removed for comparison with that of *prægrandis*.

with naked skin. There does not appear to be rigorously exercised, at least for our two Sudanese species (*Ch. boueti* and *Ch. chœrophaga*), one exclusive type of mammifer. I have seen, in fact, in a Phacochere's burrow (environs of Timbuctoo), both species of *Chœromyia*, but with *Ch. chœrophaga* incontestably predominating. If one can speak of an *absolute* specificity of the diverse species

of *Chœromyia*, either for the Orycteropes or the Phacocheres, there would be a distinctly strong *relative specificity* of *Ch. boueti* with Orycteropes, and *Ch. chœrophaga* with the Phacocheres. This relative specificity is directed also, in my opinion, by the nature of the burrow as well as the nature of the host.' Now *prægrandis*, as far as I know, is almost entirely restricted to the Aard Vark (*Orycteropus*), its distribution corresponding more or less closely with that of the several species of this ancient genus of Edentates. Dr. A. T. Hopwood, Palaeontological Department, British Museum, has very kindly given me the following notes on the genealogy of the Aard Vark. ' Aard Varks are known from the Lower Pliocene of Pikermi, near Athens, and from beds of the same age in the Isle of Samos. I have found a single tooth in lower Miocene deposits in Kenya. So far as I know the Grecian forms were burrowers and it is probable that those from Kenya were too, though there is no evidence either way.' And, later, he tells me ' that the Aard Vark line goes back a very long way indeed, and that a humerus from the Oligocene of Quercy, France, has been described as *Palaeorycteropus quercyi*.' These two species of *Auchmeromyia*, then, are more closely associated with the more ancient animal host, and the terminalia of both, like those of the three other species, are all built on a common ground-plan of structure. The phallosome of *prægrandis* has, however, undergone considerable changes from the ancestral form which is exhibited by that of the four other species, *boueti* in particular. What has brought about this change ? I believe that the answer is to be found in the structure of the end of the ♀ abdomen of *prægrandis*. The ventral edges of tergum 5 are normally closely applied (sometimes one overlaps the other), so that the stout bristles cross each other; furthermore, the ovipositor is much reduced and shortened, and the genital opening is narrower than that of the other four species, in which it is particularly wide. In order for insemination to take place, the phallosome would necessarily have to be longer in order to reach the genital opening, for it should be remembered that the ovipositor is not extended much during copulation ; and in addition the distal segments of the ninth coxites would have to be longer, wider and stouter, in order to evert the bristly edges of tergum 5. And this is exactly what has taken place : the distal segments of the ninth coxites of the ♂ *prægrandis* are admirably adapted for this purpose (fig. 18, *b*), and the phallosome has become narrower by the reduction of all flaps and the great elongation of the median shaft which carries the ejaculatory duct at its flexible end. The conclusion, then, is that the phallosome of *boueti*, *bequaerti*, *chœrophaga* and *luteola* represents the ancestral form, and that the phallosome of *prægrandis* has become modified from it, owing to the changes in the structure of the end of the ♀ abdomen, for there can, I think, be little doubt that it uses the end of the abdomen like a plough. I can, therefore, see no justification for placing *boueti*, *bequaerti*, and *chœrophaga* in a genus distinct from *luteola*, nor can I see any reason for making a new genus (*Parachœromyia* Vill.) for *prægrandis*. The five species represent a homogeneous group of very ancient Diptera whose larvae live on

the blood of man, the Aard Vark and the wart-hog, all hosts which have one character in common, viz., skins sparsely covered with hair. There can be little doubt that the Aard Vark and its ancient ancestors was the primary host, and that in more recent times (geologically) *luteola* has adapted itself to man ; *boueti* and *prægrandis* still keep more or less closely associated with the Aard Vark, but like *bequaerti* and *chœrophaga* they can also live in contact with the wart-hog, a hairless animal, which, though it can burrow, prefers ready-made burrows and commonly frequents those of the Aard Vark.

The comparative study of the ♂ and ♀ terminalia of the five known species of *Auchmeromyia* has led to the following conclusions :—

1. The structure of the anal cerci, ninth coxites, phallosome and ♀ terminalia exhibit no structural relationships with those of any Calliphorine known to me at present. The genus cannot, therefore, be retained in the subfamily Calliphorinae, in which it is usually placed, and for its reception the subfamily Auchmeromyinae is erected.

2. The anal cerci, ninth coxites, and phallosome of the five species exhibit a common ground plan of structure and are adapted to that of the ♀ terminalia. The anal cerci have become fused and greatly elongated, and have now taken on the function of clasping the end of the abdomen. The ninth coxites have lost their original function of clasping the end of the ovipositor *laterally*, and have become *everters* of the ventral edges of tergum 5 of the ♀. The phallosome of *luteola*, *chœrophaga*, *bequaerti* and *boueti* is relatively short and massive, and consists of two parts united basally by a characteristic *chitinous bar*. The phallosome of *prægrandis*, though possessing these fundamental characters—especially the division into two parts and the presence of the proximal bar—has become elongated and narrowed, in conformity with the changes in the structure of the end of the ♀ abdomen.

3. The ♀ terminalia of the five species similarly exhibit a common ground-plan of structure. The ventral edges of tergum 5 are adapted to form a plough. The ovipositor is relatively short (very short in *prægrandis*) and wide, the various sclerites are strongly developed and of a characteristic shape, and in the species with a short phallosome the signum is more or less developed. In *prægrandis* the ventral edges of tergum 5 practically overlap and are armed with strong decussating bristles, and as a result the sclerites of the ovipositor have become much reduced and narrowed.

4. The larva of *luteola* and *chœrophaga* (the only two known at present) are structurally similar in every detail, and it is assumed that the larva of each of the three other species also has this common structure.

5. The genus is a homogeneous one, and there is no valid reason for splitting it into subgenera (*Chœromyia* Roubaud, *Parachœromyia* Villeneuve), for it is clear that the species are closely related.

GENUS AUCHMEROMYIA BRAUER AND VON BERGENSTAMM (EMENDED PATTON 1935)

GENERIC CHARACTERS. LARVA. Third Stage. Cuticle devoid of obvious spines and with longitudinal and horizontal sulci ; oral hooks of equal length and with a lateral toothed cutting plate close to outer side of each ; anterior spiracles not projecting but flush with surface ; eighth segment flattened dorso-ventrally, with the usual five fleshy processes on each side ; posterior spiracular plates small, very widely separated, with three straight, short, outwardly directed breathing slits. ADULT. Males, like females, dichoptic ; arista with spinulae on both sides and about distal third bare ; palps rather club-shaped and somewhat flattened. Proboscis of normal length and structure, that is, not adapted for tearing tissues of other insects, as in *Bengalia*. Squama with or without hairs on upper surface. MALE TERMINALIA. ANAL CERCI. Fused (sometimes with bifid end) to form a median, stout, often very long, curved rod. *Ninth Coxite*. Distal segment in lateral position but migrated forwards so that ends lie on each side of base of phallosome ; varying in width and length, but generally finger-like, with end turned in ; used for everting ventral edges of tergum 5 during copulation ; proximal segment short, wide and plate-like, and joined to its fellow. PHALLOSOME. Either short and massive with characteristic serrated flaps, or very long and without flaps ; distal portion always consisting of two parts joined by membrane, and at base by a characteristic bar which forms the median shaft. FEMALE TERMINALIA. Tergum 5 with ventral edges in close apposition ; they may be armed with very strong bristles, and may almost completely cover genital sulcus, thus giving the abdomen a pointed (and sometimes turned-up) appearance ; used for making a furrow in earth for eggs. OVIPOSITOR. Short and wide, or very short ; tergal and sternal plates complete. Tergum 6 consisting of either three distinct plates, the median one small, or the median plate not completely separated. *Chitinous Plate of Uterus*. Either well developed or wanting. SPERMATHECAE. Either long and narrow, or short, wide and pear-shaped.

It is difficult to find external characters on the adults which can be used to define the genus, for practically all the chaetotactic characters are common to *Bengalia* and *Cordylobia* as well. All three genera, for instance, have the sterno-pleurals arranged 1 : 1, and the dorso-centrals and achrostichals are very variable ; so also are the hairs and bristles on the vertex. I do not consider the characters given by Surcouf to be of generic value.

SUMMARY

The comparative study of the ♂ and ♀ terminalia of the five known species of *Auchmeromyia* has demonstrated their close relationships to each other. There seems little doubt that the phallosome and ovipositor of *luteola, chœrophaga, bequaerti* and *boueti* represent the primitive form of these structures. A critical study of the phallosome of *boueti* and *prægrandis* suggests that the latter

originated from the former ; the phallosome of *prægrandis* has undergone changes (elongation and simplification) in conformity with the changes in the ventral edges of tergum **5** of the ♀ and the shortening of the ovipositor. These two species are more closely associated with the Aard Varks (*Orycteropus* spp.), a group of very ancient burrowing Edentata. *A. luteola* has probably much later (geologically) become associated with man, and does not now invade the burrows of the Aard Vark. Roubaud suggests that, when man becomes completely civilized in Africa and ceases to sleep on the ground but on a bed, *luteola* will entirely disappear ; but it may again return to its ancestral host. *A. chœrophaga*, *A. bequaerti*, *A. boueti* and even *A. prægrandis* are more or less also associated with the wart-hog (*Phacocherus* sp.), also a hairless animal, which, though it can burrow, prefers to use the ready-made burrows of the Aard Vark.

The larvae of the five species suck the blood of man, of the Aard Vark and of the wart-hog, all hosts with skins sparsely covered with hair ; they are not adapted to feed on the blood of hosts with hairy skins. The larva has a special cutting plate.

The genus is a homogeneous one, and there is no reason for splitting it into subgenera (*Chœromyia* Roubaud, *Parachœromyia* Villeneuve). It is not related to the Calliphorinae, from which subfamily it should be removed, and it is here placed in the new subfamily Auchmeromyinae.

REFERENCES

Macquart, J. (1835). Histoire naturelle des Diptères. vol. 2.

Patton, W. S. (1935). Studies on the higher Diptera of medical and veterinary importance : a revision of the genera of the subfamily Calliphorinae based on a comparative study of the male and female terminalia. The genus *Calliphora* Robineau-Desvoidy (sens. lat.) : a practical guide to the Australian species. *Ann. Trop. Med. & Parasitol.*, **29**, 19.

Robineau-Desvoidy, J. B. (1830). Essai sur les myodaires.

Roubaud, E. (1914). Études sur la faune parasitaire de l'Afrique Occidentale Française. I : Les producteurs de myiases et agents similaires chez l'homme et les animaux. Paris.

Surcouf, J. M. R. (1920*a*). Revision des Muscidae Testaceae. *Nouv. Arch. du Mus. d'Hist. Nat.*, sér. 6.

——— (1920*b*). Sur une critique récente de J. Villeneuve. *Bull. Soc. Entom. France*, 249.

Villeneuve, J. (1920). À propos de la revision des Muscidae Testaceae de J. Surcouf. *Ibid.*, 223.

TRYPANOCIDAL ACTION OF TWO ARSENICALS, K.324 AND K.352, ON INFECTIONS IN MICE AND RABBITS

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Object of Investigation

The trypanocidal activity of a long series of aromatic arsenical compounds, prepared by Dr. H. King and his colleagues, has been studied during a number of years. The preliminary results (Cohen, King and Strangeways, 1931, 1932) obtained recently with two of these compounds indicated that they were exceptionally active in curing *Trypanosoma equiperdum* infections in mice. The present investigation, therefore, was undertaken to find out whether these results were confirmed when larger numbers of animals were used as well as different species of trypanosomes.

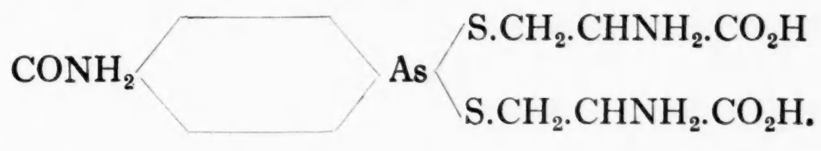
Further experiments have also been made to test the therapeutic value of the two compounds when used for the treatment of trypanosomiasis in rabbits. This is more nearly analogous to the human condition, since there is definite tissue involvement rather than a blood infection, as is the case with mice.

Control of Method

It was necessary to control in some way the methods of investigation, especially in the case of the rabbit infections where the disappearance of clinical symptoms was used as a criterion of cure. Parallel tests, therefore, were made with tryparsamide, this drug having a known therapeutic value both in animal and human trypanosome infections.

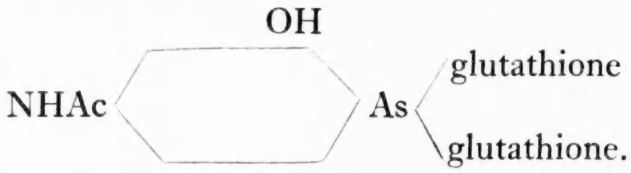
Preparation of Solutions

1. *K.324.* Di (β-carboxy-β-aminoethyl) benzamide-p-thioarsinite.



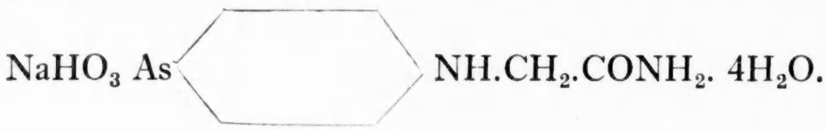
The required amount of compound was weighed accurately and saturated sodium bicarbonate solution added in just sufficient quantity to dissolve the powder when briefly warmed in a boiling-water bath. Caustic alkali must be avoided. About 0·75 c.cm. of saturated sodium bicarbonate solution was required for 0·05 gm. of K.324. This solution was then cooled rapidly and made up to the required amount with freshly distilled water.

2. *K.352.* Di glutathionyl-4-acetamino-2-hydroxyphenyl thioarsinite.



The required weight of compound was dissolved in freshly distilled water and saturated sodium bicarbonate solution was added to neutralize. About 3–4 drops of sodium bicarbonate solution were required for 0·1 gm. K.352. The neutral solution was then made up to the required strength.

3. *Tryparsamide.* Phenylglycineamide-p-arsonic acid (sodium salt).



A weighed amount of this compound was made up to the required strength with freshly distilled water.

MOUSE TESTS

Toxicity Tests

Toxicity tests on mice were made with single intravenous injections of drug only, to find the dose of each compound tolerated by a high percentage of treated animals. The mice were kept under observation for one month after treatment, in order to detect any late symptoms of undue toxicity, such as the involvement of the central nervous system. Deaths due to the injection of drug always occurred within the first few days.

Injections were made slowly, since the sudden introduction even of comparatively low doses of both K.324 and K.352 produced symptoms of undue toxicity in mice. These were apparent as an increased rate of respiration, ruffling of the fur, and, with higher doses, watering of the eyes. It was also important to have the solutions as nearly neutral as possible, since excessive alkalinity produced similar toxic symptoms.

Toxicity of K.324

	No. of mice	Dose	Result
Test 1	5	0·04 mgm. per gm. 0·2 per cent. solution	5 mice survived
	5	0·05 " " " " "	5 " "
	5	0·075 " " " " "	5 " "
	5	0·1 " " " " "	5 " "
Test 2	5	0·1 " " 0·4 " "	5 " "
	5	0·16 " " " " "	2 " "
Test 3	30	0·075 " " 0·5 " "	25 " "
	30	0·1 " " " " "	18 " "
	30	0·15 " " " " "	1 mouse "

Maximum tolerated dose = 0·075 mgm. per gm.

Toxicity of K.352

	No. of mice	Dose	Result
Test 1	5	0·1 mgm. per gm. 1·0 per cent. solution	5 mice survived
	5	0·15 ,, ,, ,, ,, ,,	5 ,, ,,
	5	0·2 ,, ,, ,, ,, ,,	4 ,, ,,
	5	0·25 ,, ,, ,, ,, ,,	2 ,, ,,
Test 2	5	0·3 ,, ,, ,, ,, ,,	3 ,, ,,
	5	0·35 ,, ,, ,, ,, ,,	2 ,, ,,
Test 3	30	0·2 ,, ,, ,, ,, ,,	29 ,, ,,
	30	0·3 ,, ,, ,, ,, ,,	17 ,, ,,
	20	0·4 ,, ,, ,, ,, ,,	4 ,, ,,

Maximum tolerated dose = 0·2 mgm. per gm.

Toxicity of Tryparsamide

	No. of mice	Dose	Result
Test 1	5	2·0 mgm. per gm. 10 per cent. solution	5 mice survived
	5	3·0 ,, ,, ,, ,, ,,	5 ,, ,,
	4	4·0 ,, ,, ,, ,, ,,	1 mouse ,,
Test 2	10	3·0 ,, ,, ,, ,, ,,	9 mice ,,
	10	3·5 ,, ,, ,, ,, ,,	9 ,, ,,*

* 5 mice showed symptoms of a toxic action, but recovered after 3 days.

Maximum tolerated dose = 3·0–3·5 mgm. per gm.

Summary. The maximum dose which can be given to mice in a single intravenous injection of the three drugs are 0·075 mgm. per gm. K.324, 0·2 mgm. per gm. K.352 and 3·0–3·5 mgm. per gm. tryparsamide.

Therapeutic Tests

The therapeutic values of K.324 and K.352 have been tested on the infections produced in mice by five different species of trypanosomes. These fall under two headings :

(*a*) Trypanosomes producing rapidly fatal infections in untreated mice :

- (1) *T. equiperdum.*
- (2) *T. rhodesiense.*
- (3) *T. brucei.*

(*b*) Trypanosomes producing chronic, though usually fatal infections in untreated mice :

- (1) *T. gambiense.*
- (2) *T. congolense.*

The rapidly fatal strains used for comparative tests were all apparently constant in their virulence for mice and in their reactions to drugs. Except for *T. equiperdum*, which had been passaged mainly through rats for a number of years, they had all been passaged through mice long enough to produce a constant infection in these animals. More exact details of the histories of the different strains tested are given under the appropriate headings. Details of the means of propagation of the two ' chronic ' species of trypanosomes are also given later.

Experimental Method

The method adopted for studying the therapeutic values of the different compounds is based on that employed for the routine testing of neoarsphenamine.

Preparation of infected mice. Healthy mice were given intraperitoneal inoculations of suspensions in citrate saline of the heart blood from an animal highly infected with the required strain of trypanosome. The amount and strength of the inoculum varied somewhat with the different strains and will be given in detail for each of these.

Injections of drugs, always made intravenously into the tail veins of the infected mice, were given at an interval after inoculation when there was a blood infection which varied between 50,000 and 500,000 trypanosomes per c.mm. of blood. The degree of infection in each mouse was usually counted by means of a Thomas-Zeiss haemocytometer, using as stain and diluent Toisson's fluid containing about 1 per cent. formol. At other times the degree of infection was examined directly by the removal of a drop of blood from the tail.

After examination, the mice were arranged in groups for testing, the number of groups depending on the number of doses of drugs to be tested. Since all the mice did not show the same number of circulating parasites, every effort was made to distribute them in comparable groups.

In the case of *T. congolense*, it was not possible to obtain any idea of the extent of infection in the general circulation by examination of peripheral blood. It was often found that an animal which had shown a rich infection of trypanosomes in the tail blood showed very few in that obtained from the heart after death. This fact has been reported by Browning, Cappell and Gulbransen (1934), and is said by them to be due to the stasis produced in the peripheral circulation by *T. congolense* infections.

Observation of animals. The mice were examined daily for peripheral infection by the removal of a drop of blood from the tail. They were kept under observation for lengths of time after treatment which varied with the species of trypanosome. In the case of the three rapidly fatal infections, *T. equiperdum*, *T. rhodesiense* and *T. brucei*, a ' permanent ' cure was assumed after a mouse had remained free from peripheral blood infection for 30 days. Many experiments, however, were continued for a further two to eight weeks.

The chronic character of *T. gambiense* and *T. congolense* infections necessitated longer examination of treated animals.

The results obtained are tabulated briefly in the case of *T. equiperdum*, *T. rhodesiense* and *T. brucei*, but the nature of the results obtained with *T. gambiense* and *T. congolense* made more detailed descriptions necessary. Mice dying from intercurrent bacterial infections during the course of an experiment are excluded from the Tables.

Results obtained with rapidly fatal infections *T. equiperdum*, *T. rhodesiense* and *T. brucei*

1. *Trypanosoma equiperdum*

History of strain. The exact origin of this strain of trypanosome is unknown. It was sent to Hampstead some 10–15 years ago by Professor Mesnil of the Pasteur Institute, and has been kept since then mainly in rats. Guinea-pigs have been used as an additional reservoir, since the infection in them is fatal only after 3–4 months. For purposes of test the strain was passaged through rats and from these animals into mice.

Details of inoculation. Mice to be used for drug treatment were inoculated intraperitoneally with a suspension in citrate saline of heart blood from an infected rat. This was diluted to contain 7,000 trypanosomes per c.mm. of suspension and 0·5 c.cm. was given to each mouse. The animals were ready for treatment two days later.

2. *Trypanosoma rhodesiense*

History of strain. The strain came originally from a patient in the London School of Hygiene and Tropical Medicine and was sent to Professor Warrington Yorke in June, 1924. It was passaged through mice and guinea-pigs until August, 1928, since when it has been maintained in mice only. It was sent to Hampstead on February 9th, 1933.

Details of inoculation. Mice to be used for drug treatment were inoculated intraperitoneally with a suspension in citrate saline of heart blood from an infected mouse. This was diluted to contain 5,000 trypanosomes per c.mm. of suspension and 0·2 c.cm. was given to each mouse. The animal was ready for treatment two days later.

3. *Trypanosoma brucei*

History of strain. This strain was obtained originally from a naturally infected dog in South Ankole, Uganda. It was passaged through guinea-pigs for 8 months before being sent to Professor Browning, who propagated it in mice for 10½ months before November 30th, 1932, when it was sent to Hampstead.

The above strain was used for all tabulated experiments with K.324 and K.352, but it was lost before the tests with tryparsamide had been performed. These were made, therefore, on a strain of *T. brucei* of the same origin, which

TABLE I

	Dose (mgm. per gm.)	*T. equiperdum* No. of mice	*T. equiperdum* No. of mice permanently cured	*T. equiperdum* Percentage of permanent cures	*T. rhodesiense* No. of mice	*T. rhodesiense* No. of mice permanently cured	*T. rhodesiense* Percentage of permanent cures	*T. brucei* No. of mice	*T. brucei* No. of mice permanently cured	*T. brucei* Percentage of permanent cures
	0·002 to 0·0025	—	—	—	—	—	—	20	3	15
K.324	0·003 to 0·004	—	—	—	—	—	—	27	13	48
	0·005	33	10	33	19	3	16	39	17	44
Max. tol. dose	0·0075	21	14	67	7	4	57	5	5	100
= 0·075 mgm./gm.	0·01	38	22	58	18	17	94	19	19	100
	0·015	37	28	76	—	—	—	—	—	—
	0·02	34	23	68	—	—	—	—	—	—
	0·025	12	11	92	—	—	—	—	—	—
	0·0025	—	—	—	—	—	—	10	0	0
K.352	0·003 to 0·004	—	—	—	20	0	0	20	3	15
	0·005	23	3	13	23	1	4	19	10	53
Max. tol. dose	0·0075	10	7	70	10	5	50	17	17	100
= 0·2 mgm./gm.	0·01	54	34	63	35	30	86	9	9	100
	0·02	15	13	87	10	10	100	—	—	—
	0·03 to 0·04	11	11	100	—	—	—	—	—	—
Tryparsamide	0·25	—	—	—	—	—	—	10	0	0
	0·4 to 0·5	10	1	10	30	1	3	10	1	10
Max. tol. dose	0·75	9	3	33	19	4	21	9	8	91
= 3·5 mgm./gm.	1·0	10	7	70	8	7	88	8	8	100
	1·5	10	7	70	7	7	100	—	—	—

had been propagated in guinea-pigs by Dr. Wenyon up to the time when it was sent to Hampstead, November 24th, 1932. Comparative tests made in June, 1933, had shown that the responses of the two strains to treatment with K.324 were identical. There seems no doubt, therefore, that the experiments made with tryparsamide in July and September, 1934, can be compared with those made with the other two compounds using the original mouse strain of *T. brucei.*

Details of inoculation. These are the same as those given for *T. rhodesiense.*

Results

Table I summarizes the results obtained with K.324, K.352 and tryparsamide when tested on the infections produced by the three species of trypanosome, *T. equiperdum*, *T. rhodesiense* and *T. brucei.* Mice which were ' permanently ' cured usually showed no trypanosomes in the peripheral circulation 24 hours after treatment.

Table II summarizes the results in Table I by selecting the approximate dose of each compound which will produce a high percentage (80 per cent. or more) of cures in mice infected with any of the three species of trypanosome. Included in this Table, also, are the approximate therapeutic indices of each drug for the different species of trypanosome. The therapeutic index, I, is represented as the ratio of the *minimum curative dose* (M.C.D.) to the *maximum tolerated dose.*

TABLE II

Drug	*T. equiperdum* M.C.D. (mgm. per gm.)	*T. rhodesiense* M.C.D. (mgm. per gm.)	*T. brucei* M.C.D. (mgm. per gm.)
K.324	0·015–0·025 (I = 1/3·75)	0·01 (I = 1/7·5)	0·0075 (I = 1/10)
K.352	0·02 (I = 1/10)	0·01 (I = 1/20)	0·01 (I = 1/20)

Conclusions Arrived at from Results Obtained

The following is a summary of the conclusions which can be drawn from the above results.

1. The two aromatic arsenicals K.324 and K.352 provide efficient means of curing infections in mice due to three rapidly fatal trypanosome infections, *T. equiperdum*, *T. rhodesiense* and *T. brucei.*

2. Infections produced by the three species vary somewhat in the ease with which they can be cured in mice. Thus, *T. brucei* is the most easily cured, *T. rhodesiense* rather less so, while *T. equiperdum* requires relatively large doses of K.324, K.352 or tryparsamide to produce permanent cures. Similar relative differences in sensitivity have been observed by other workers.

3. The results obtained with *T. equiperdum* using different doses of drug are far more irregular than those obtained with either *T. brucei* or *T. rhodesiense*. The cause of the observed irregularities may, possibly, be due to the method of propagation of the strain of trypanosomes, although the necessary experiments to prove this have not been undertaken.

Results obtained with 'chronic' infections *T. gambiense* and *T. congolense*

1. *Trypanosoma gambiense*

History of strain. The strain of *T. gambiense* was obtained from a patient in Entebbe (Uganda) in April, 1931. It was sent to Professor Warrington Yorke, who passaged it through 3 guinea-pigs and 3 mice before it was sent to Hampstead on November 25th, 1932. Since then it has been propagated in mice only.

Nature of untreated infections. The virulence of this strain of *T. gambiense* for mice seems to vary a great deal for different animals. Whereas some mice die within the comparatively short period of a week to a month after inoculation, others may live as long as 8 to 9 months without showing any signs of trypanosome infection after the first week. At first these mice were discarded as 'spontaneous cures,' but recently this has been found to be a false assumption. A mouse, inoculated on February 6th, 1934, for purposes of passage, remained perfectly healthy and apparently free from trypanosome infection up to December 4th, 1934, when it was killed. A number of drops of blood were examined for trypanosomes immediately before death, and after death 1·0 c.cm. of blood was removed by heart puncture and inoculated intraperitoneally into a clean mouse. No trypanosomes were found, but the inoculated mouse became highly infected 28 days later. Further examination was made of a smear from the circulation to the brain as near as possible to the choroid plexus. This showed definite trypanosome infection, as many as 5 lively trypanosomes being found in the moist smear examined under the microscope. Washings from the skull and brain were inoculated intraperitoneally into 3 mice. The latter were examined frequently up to December 28th, 1934, i.e., 24 days after inoculation. They remained healthy and no trypanosomes were found. Four days later, however, all three mice were dead. Post-mortem changes were too far advanced for the cause of death to be diagnosed, though it was probably due to trypanosome infection.

A second case of brain infection in an apparently healthy mouse is reported in Experiment 2 below. In this case the mouse had been treated with K.352 and was killed 8 months later. Trypanosomes were found in a moist smear from the brain, and sections cut through the choroid plexus showed definite round-cell infiltration round a vein running through the centre.

Further material for study is not yet available, but it seems probable that the choroid plexus is a seat of *T. gambiense* infection in mice and that animals can harbour the trypanosomes here without showing any signs of the disease.

Details of inoculation. Mice were inoculated intraperitoneally with 0·5 c.cm. of a suspension of the heart blood from an infected mouse, containing about 20,000 trypanosomes per c.mm. This, however, was not counted accurately. The animals were left 2–3 days before treatment, when they showed the required degree of infection in the peripheral blood.

Observation of animals. The chronic character of the untreated infections of *T. gambiense* made it necessary to keep the mice under observation for as long as possible. This varied from 4–9 months, except in one experiment, in which some of the mice were discarded after 2 months because of intercurrent infections. Daily examinations (6 times a week) of drops of peripheral blood were made during the early part of all experiments, but, later, examinations were made only once to three times weekly.

Presentation of Results

Brief descriptions of each of the four experiments performed with this species of trypanosome are given below.

Experiment 1

Drugs used. K.324, K.352.
Details of test. Mice inoculated 2 days before treatment.
Examined daily for 1 month and at intervals for 8 months longer. *Total = 9 months.*

Drug	No. of mice	Dose (mgm. per gm.)	Result
K.324	5	0·1	5 mice remained negative throughout.
K.352	5	0·1	5 ,, ,, ,, ,,
Controls	10	—	10 mice died with positive blood infection in 8-25 days.

Experiment 2

Drug used. K.352.
Details of test. Mice inoculated 2 days before treatment.
Examined (*a*) daily for 1 month, (*b*) at intervals for 3½ months, (*c*) daily for 1 month, (*d*) at intervals for 3 months. *Total = 8 months.*

No. of mice	Dose (mgm. per gm.)	Result
4	0·05	4 mice remained negative throughout.
5	0·04	5 ,, ,, ,, ,,
5	0·03	4 ,, ,, ,, ,,
		1 mouse positive 4½ months (145th day) but negative and well until end of experiment.
		P.M. *Heart blood* (7 drops). No trypanosomes seen.
		Brain. Trypanosomes seen in moist smear.
		Sections of Brain. Infiltration round vein in choroid plexus.
5	0·02	5 mice remained negative throughout.
20	Controls	20 ,, died with positive blood infection 5-34 days.

Experiment 3

Drugs used. K.324, K.352, tryparsamide.
Details of test. Mice inoculated 3 days before treatment.
Examined daily for 2 months and at intervals for 4 months longer. *Total = 6 months.*

Drug	No. of mice	Dose (mgm. per gm.)	Result
K.324	3	0·02	3 mice remained negative throughout.
	5	0·01	3 „ „ „ „
			1 mouse died (negative) 3 months.
			1 „ positive 7th day, negative until end of experiment.
	5	0·005	2 mice remained negative throughout.
			1 mouse died (negative) $2\frac{1}{2}$ months.
			1 „ relapse 14th day, died 38th day.
			1 „ „ 22nd „ „ 113th „
K.352	5	0·01	4 mice remained negative throughout.
			1 mouse died (negative) 2 months.
	5	0·005	1 mouse remained negative throughout.
			1 „ died (negative) 45th day.
			3 mice relapsed 15th-24th day, died 35th-63rd day.
Tryparsamide	5	1·5	4 „ remained negative throughout.
			1 mouse relapsed 3 months and died 1 month later.
	5	0·75	5 mice remained negative throughout.

Experiment 4

Drugs used. K.324, K.352.
Details of test. Mice inoculated 3 days before treatment.

All were examined daily for 1 month, and at intervals for a further 26 days in the case of K.324, when the experiment had to be abandoned on account of an intercurrent bacterial infection. The mice treated with K.352 were examined for 5 months.

Drug	No. of mice	Dose (mgm. per gm.)	Result
K.324	9	0·005	4 mice remained negative (46 days).
			5 „ relapse 10th-45th day—all alive 46th day.
K.352	10	0·01	8 „ remained negative throughout (5 months).
			2 „ died (negative) 39th day and 4 months.
	9	0·005	4 „ remained negative throughout (5 months).
			1 mouse died (negative) 4 months.
			4 mice relapse 25th-62nd day, 3 died 59 days—$4\frac{1}{2}$ months.
			1 still alive 5 months.

Conclusions

The following conclusions can be drawn from an analysis of the results obtained in the above experiments.

1. Doses of 0·01 mgm. per gm. and more of both K.324 and K.352, and of 0·75 mgm. per gm. tryparsamide will clear the peripheral blood of *T. gambiense* infections for long periods.

2. Relapses are common after treatment with 0·005 mgm. per gm. of both K.324 and K.352.

3. A negative result in examination of peripheral blood does not necessarily indicate freedom from *T. gambiense* infection. Trypanosomes may be present in the brain circulation (choroid plexus) without any outward signs of infection on the part of the mouse.

2. *Trypanosoma congolense*

This species of trypanosome is well known to be practically incurable with any of the ordinary arsenical compounds, but it was thought worth while to discover whether the compounds under test had any effect on it.

History of strains. Two strains of *T. congolense* have been tested in mice.

1. *T. congolense B.* This strain was sent to Professor Browning from Tanganyika Territory. It was propagated in mice for 3 years before it was sent to Hampstead on November 30th, 1932.

2. *T. congolense N.* This was isolated from an infected goat in Tanganyika Territory in November, 1933. It was inoculated into guinea-pigs for transport to England, and has been kept at Hampstead both in mice and guinea-pigs since December 8th, 1933. The strain had, therefore, been propagated artificially only a few months before it was used for testing the two drugs.

Nature of untreated infections. Both strains of *T. congolense* gave rise to chronic infections in untreated mice, especially *T. congolense B.* Spontaneous cures occurred with both strains, making their propagation in mice relatively difficult. The strain of *T. congolense N.* maintained in guinea-pigs killed these animals in 2-3 weeks. Spontaneous cures have not yet been observed.

Inoculation of animals. Mice were inoculated intraperitoneally with a suspension of heart blood from an infected mouse. The suspension contained about 1 trypanosome per microscopic field, and 0·5 c.cm. was given to each mouse. The animals were kept 6–11 days before treatment, when a definite infection was apparent in the peripheral blood stream.

Presentation of Results

The results of individual experiments are given below in the same way as those for *T. gambiense*.

Experiment 1

T. congolense B.

Drug used. K.352.

Details of test. Mice inoculated 11 days before treatment.

Examined daily.

No. of mice	Dose (mgm. per gm.)	Result
3	0·1	3 mice cleared of infection for 15-25 days, all relapsed and dead 22-27 days after treatment.
6	0·05	6 mice cleared of infection for 4-14 days. Dead 14-22 days after treatment.
6	0·025	6 mice cleared of infection for 1-2 days. Died 9-35 days after treatment.
18	Control	18 mice dead 12 days to 3 months after inoculation.

Experiment 2

T. congolense B.

Drugs used. K.324, tryparsamide.

Details of test. Inoculated 8 days before treatment.

Examined daily for 4 months and then at intervals for a further 2 months. *Total = 6 months.*

Drug	No. of mice	Dose (mgm. per gm.)	Result
K.324	7	0·075	7 mice cleared of infection for 2-8 days. All dead 9 days to 4 months after treatment.
	8	0·05	3 mice cleared of infection for 1-2 days. 5 „ not cleared. 7 mice dead 6-10 days after treatment. 1 still alive but infected 6 months after treatment.
Tryparsamide	8	2·0	5 mice cleared of infection for 2-4 days, 3 not cleared. 5 mice dead 9-13 days, 3 dead 1½-6 months after treatment.
	8	1·5	2 mice cleared of infection for 3-7 days. 6 „ not cleared. 7 mice dead 3-30 days after treatment. 1 mouse still alive, though infected, 6 months after treatment.

Experiment 3

T. congolense N.

Drug used. K.324. *Repeated injections.*

Details of test. Inoculated 6 days before treatment.

Examined daily.

14 mice. All given 0·02 + 0·04 mgm. per gm. (2 doses in 5 hrs.) on 6th day after inoculation.

1. 5 mice no further treatment. 5 dead 7th–10th day.
2. 4 mice 0·05 mgm. per gm. 7th day. 4 dead 9th–10th day.
3. 5 mice 0·05 mgm. per gm. 7th day + 0·075 mgm. per gm. 9th day. 5 dead 10th–55th day.

There was no reduction in peripheral infection throughout.

5 mice. Controls. No treatment. 5 mice dead 7th–12th day.

Experiment 4

T. congolense N.

Drugs used. K.324, K.352 (repeated doses), tartar emetic.

Details of test. Mice inoculated 7 days before treatment.

Examined daily for 7 days after treatment, when experiment was discontinued.

Drug	No. of mice	Dose (mgm. per gm.)	Result
K.324	7	0·05. 2 doses, with 2 days' interval	*No reduction* in peripheral infection.
K.352	8	0·075. 2 doses, with 2 days' interval	„ „ „ „
Tartar emetic	7	0·03. 1 dose	*7 mice cleared* of peripheral infection for 2-5 days.

Conclusions

No curative value is apparent for either K.324 or K.352 in *T. congolense* infections in mice.

RABBIT TESTS

Methods of Investigation

The methods employed for studying the therapeutic values of the two compounds K.324 and K.352 were based on those reported by Pearce and Brown (1918, 1919, 1921). More exact details of the method of testing were obtained from Professor Warrington Yorke, who kindly demonstrated them at the beginning of this investigation.

Toxicity Tests

Apart from a few preliminary tests with single intravenous injections of drug, no attempt was made to find accurately the toxicity of the two compounds for normal rabbits. The preliminary tests indicated what doses of each drug could be administered with safety. It was thought that any toxic effects of the two compounds could be studied better during the course of treatment of infected animals.

Injections were always made comparatively slowly, but no excessive care was taken. In spite of this, no signs of any immediate toxic effects, similar to those produced in mice, were apparent, even after the injection of doses which later killed the rabbit or produced temporary loss in weight.

The effect of administration of a number of small doses of each drug, as well as of tryparsamide, was studied in a series of animals. These were given intravenous injections three times weekly over a period of 3–5 weeks. The results are included in the Tables for each drug, dealing with the therapeutic effect of repeated small doses.

Therapeutic Tests

Trypanosome infection. One species of trypanosome, only, was used for testing the therapeutic efficiency of the two compounds in rabbit infections. This was the same strain of *T. rhodesiense* as that tested in mice. It was a strain adapted to mouse passage over a number of years, and would not, therefore, be expected to give results similar to those obtained by Pearce and Brown (1921) with one more recently isolated.

Inoculations were made intravenously of 1·0 c.cm. per Kg. body weight of a suspension of the heart blood from an infected mouse. The suspension contained about 10,000 trypanosomes per c.mm., but this was not counted accurately.

Time of drug injection. Injections were made intravenously 20–25 days after the inoculation of trypanosomes. After this time there were pronounced oedematous lesions on the ears and eyelids, as well as on the external genitalia. Trypanosomes could readily be found either in the peripheral circulation or in the fluid from one of these lesions.

Observation of animals. The rabbits were examined frequently after treatment and kept for periods of from 6 to 12 months before being discarded as cured. At least once during this time mice were inoculated with 1·0 c.cm. of peripheral blood from the ear vein, to test for any blood infection.

The weights of all rabbits were kept throughout, as an additional check on the health of the animals and to detect any ill effects caused by injections of the drugs, which might not otherwise be apparent. Young growing rabbits weighing 1 to 1½ Kg. were used, and adverse effects of drug administration were made

apparent either by a loss in weight or by a slowing down of the rate of gain in weight.

Effects of drug administration. The beneficial effects of the administration of effective doses of both K.324 and K.352 were apparent soon after injection. This was most pronounced in the ears, the inflammation in which was gone, or nearly gone, the day after injection. Local oedema was correspondingly diminished, and crusty lesions became dry, with healing scabs, in a short time.

Comparison with the effects of a curative dose of tryparsamide was rather difficult, but there were indications that the beneficial effects of the two new compounds were more rapidly apparent, although not sufficiently so to make a dogmatic statement possible.

Loss in weight after treatment. When loss in weight occurred it was usually from 100 to 400 gm. within a few days, in a rabbit weighing $1\frac{1}{2}$ to 2 Kg. A gain in weight then followed, and by the end of a week or 10 days the weight of the animal was once more normal. In some cases there was no actual loss in weight, but merely a failure to gain weight for a few days.

Tabulation of Results

The results obtained with individual rabbits are tabulated briefly, the animals being arranged in order of increasing size of the initial dose. In most cases, if death did not intervene, treatment was continued until a cure was effected, unless the clinical condition of an animal was considered too bad to warrant further treatment.

Results are given of two types of treatment with each drug :—

1. Single injections of small or large doses.
2. Repeated injections of small doses.

Except for rabbits 72 and 74 (Table IV), no attempts have been made to produce cures by longer courses of treatment with very small doses of the two drugs. This could probably be effected, although the possibility of the production of arsenic resistance by such treatment cannot be excluded.

TABLE III

A. K.324 (1) Single Doses

Serial no. of rabbit	Initial dose (gm. per Kg.)	Later doses (gm. per Kg.)	Result of treatment
27	0·005	—	Lesions not cleared. DIED 24 days.
29	0·005	—	Lesions cleared, *relapse*. KILLED 31 days.
51	0·01	—	Lesions cleared. *Nervous symptoms* 48 days. KILLED. Round-cell infiltration in brain. Trypanosomes seen in sections of brain. Blood infection positive.
52	0·01	—	Lesions cleared. DIED 24 days. Convulsions—no signs of infection.
25	0·01		Lesions cleared. *Relapse* 1 month.
		0·015 *(48)	Slight loss in weight. CURED 10 months.
50	0·01		Lesions cleared. *Relapse* 2½ months.
		0·02 (83)	Loss in weight. Lesions cleared. *Relapse* 3 months.
		0·03 (85)	Loss in weight. DIED 8 months. CURED.
37	0·015	—	Slight loss in weight. CURED 10 months.
38	0·015	—	Loss in weight. CURED 10 months.
48	0·015	—	Slight loss in weight. CURED 9½ months.
49	0·015		Loss in weight. Lesions cleared. *Relapse* 1½ months.
		0·02 (53)	Loss in weight. Lesions not cleared.
		0·02 (30)	Loss in weight. Lesions cleared. *Relapse* 1½ months.
		0·02 (46)	Loss in weight. CURED 12 months.
39	0·02	—	Loss in weight. CURED 10½ months.
66	0·02	—	No real loss in weight. CURED 14 months.
79	0·02		Very slight loss in weight. Lesions cleared, *relapse* 3 weeks.
		0·005, 0·01, 0·01 (28)	3 doses in 5 days. CURED 13 months.
86, 87, 88	0·03		DIED from toxic effects of drug. 86 and 87 pneumonia, 88 diarrhoea.

* N.B. Figures in parenthesis under a dose of drug represent the number of days which had elapsed since the previous injection.

Conclusions

1. Single doses of K.324 up to 0·02 gm. per Kg. can be administered with safety to rabbits infected with *T. rhodesiense*. There is a temporary loss in weight in rabbits given 0·015 gm. per Kg. and more.

2. Permanent cures can be produced with 0·015 and 0·02 gm. per Kg.

TABLE IV

A. K.324. (2) Serial Doses

Therapeutic Tests

Serial no. of rabbit	Number of doses (gm. per Kg.)	Length of course (days)	Result of treatment
72	5 × 0·005 3 × 0·01	10 5	Lesions cleared. *Relapse* 4½ months. Also 0·01 gm./Kg. *K.352*, by mistake, 9 days after last injection K.324. CURED. Still well 9½ months.
74	9 × 0·005	18	„ „ 13 „
89	3 × 0·01	6	„ „ 8 „
90	3 × 0·01	6	„ „ 8 „
91	3 × 0·01	6	„ „ 8 „
92	6 × 0·01	16	„ „ 8 „
93	6 × 0·01	16	„ „ 8 „
94	6 × 0·01	16	„ „ 8 „
95	6 × 0·01	16	„ „ 8 „
79	0·005 + 2 × 0·01	5	After failure to cure with 0·02 gm./Kg. See previous Table. CURED 13 months.

Toxicity Tests

Serial no. of rabbit	Number of doses (gm. per Kg.)	Length of course (days)	Result of treatment
111	15 × 0·01	33	No toxic effects. Gain in weight throughout.
113	15 × 0·01	33	„ „ „ „ „
110	11 × 0·015	24	„ „ „ „ slow and irregular.
112	11 × 0·015	24	„ „ „ „ „ „
			N.B. All the above 4 rabbits are alive and healthy 6 months after the last dose.

Conclusion

Short courses of treatment with 3 to 6 doses of 0·01 gm. per Kg. K.324 will produce permanent cures in rabbits infected with *T. rhodesiense.* Longer

courses of treatment with the same dose produce no toxic symptoms in normal rabbits.

TABLE V

B. K.352. (1) Single Doses

Serial no. of rabbit	Initial dose (gm. per Kg.)	Later doses	Result of treatment
33	0·004		Lesions not cleared.
		0·01 *(15)	Slight loss in weight. Lesions cleared. Relapse 1 month.
		0·02 (37)	,, ,, CURED 10 months.
32	0·004		Lesions not cleared.
		0·01 (15)	,, ,, ,,
		0·03 (12)	,, ,, ,, KILLED 8 days later.
30	0·008		,, ,, ,,
		0·01 (15)	,, ,, ,, when *accidentally killed* 20 days later.
31	0·008		,, ,, ,,
		0·01 (15)	Lesions cleared. *Relapse* 1 week.
		0·03 (12)	Check in gain of weight. CURED 9 months.
57	0·01		Lesions cleared. *Relapse* 3 weeks.
		0·02 (21)	Slight loss in weight. CURED 9 months.
58	0·01		Lesions cleared. *Relapse* $2\frac{1}{2}$ weeks.
		0·02 (18)	,, ,, ,, 2 ,,
		0·03 (37)	Loss in weight. Lesions cleared. *Relapse* 3 weeks.
		0·04 (34)	,, ,, CURED 7 months.
62	0·01		Lesions not entirely cleared.
		0·01 (18)	,, ,, cleared.
		0·01 (10)	,, better but not cleared.
		0·03 (4)	Slight loss in weight. CURED. Still well 14 months.
63	0·01		Lesions not entirely cleared.
		0·01 (18)	,, ,, ,,
		0·01 (10)	,, ,, ,,
		0·03 (4)	Slight loss in weight. CURED. Still well 14 months.

* N.B. Figures in parenthesis under a dose of drug represent the numbers of days which had elapsed since the previous injection.

TABLE V—(*continued*)

B. K.352. (1) Single Doses

Serial no. of rabbit	Initial dose (gm. per Kg.)	Later doses	Result of treatment
65	0·01	 0·01 (*18) 0·015 (10) 0·03 (4)	Lesions not entirely cleared. ,, ,, ,, ,, ,, ,, ,, not cleared. KILLED 1 month later.
55	0·015	 0·02 (20)	,, not entirely cleared. ,, cleared but rabbit developed paralysis of hind legs. KILLED 9 days later. *Round-cell infiltration of brain.* Trypanosomes found in sections of brain.
56	0·015	0·02 (25)	Slight loss in weight. Lesions cleared. *Relapse* 3 weeks. CURED 8½ months.
40	0·02		Slight loss in weight. CURED 10½ months.
41	0·02		,, ,, ,, 10½ ,,
80	0·02		,, ,, ,, Still well 14 months.
54	0·02		Loss in weight. DIED. Convulsions 6½ months. No brain infection. CURED.
53	0·02	0·02 (18)	Loss in weight. Lesions cleared. *Relapse* 2½ weeks. CURED 9 months.
82	0·02	5 × 0·01 (23)	Slight loss in weight. Lesions cleared. *Relapse* 3 weeks. 5 doses in 10 days. CURED. Still well 13 months.
42	0·03		Loss in weight. DIED convulsions and diarrhoea 3 weeks later.
43	0·03		Loss in weight. CURED 10½ months.
44	0·04		DIED 2 days later.
96	0·04		Loss in weight. CURED. Still well 8 months.
97	0·04		,, ,, ,, ,, 8 ,,
98	0·04		,, ,, ,, ,, 8 ,,

* N.B. Figures in parenthesis under a dose of drug represent the number of days which had elapsed since the previous injection.

Conclusions

1. Single doses of K.352 up to 0·04 gm. per Kg. can be administered with safety to rabbits infected with *T. rhodesiense*. There is a temporary loss in weight in animals given 0·02 gm. per Kg. and more.

2. Permanent cures can be produced with 0·02–0·04 gm. per Kg. K.352.

TABLE VI

B. K.352. (2) Serial Doses

Therapeutic Tests

Serial no. of rabbit	Number of doses (gm. per Kg.)	Length of course (days)	Result of treatment
99	4 × 0·01	8	CURED. Still well 8 months.
100	4 × 0·01	8	„ „ 8 „
101	4 × 0·01	8	„ „ 8 „
102	8 × 0·01	18	„ „ 8 „
103	8 × 0·01	18	„ „ 8 „
104	8 × 0·01	18	„ „ 8 „
82	5 × 0·01	10	After failure to cure with 0·02 gm. per Kg. See previous Table. CURED 13 months.

Toxicity Tests

Serial no. of rabbit	Number of doses (gm. per Kg.)	Length of course (days)	Result of treatment
116	15 × 0·01	33	No toxic effects. Rabbit gained weight throughout.
117	15 × 0·01	33	„ „ „ „ „
114	11 × 0·02	24	„ „ Gain in weight possibly a bit slower.
115	11 × 0·02	24	„ „ „ „ „ „ „

N.B. All the above rabbits are alive and healthy 6 months after the last injection.

Conclusion

Short courses of treatment with 4–8 doses of 0·01 gm. per Kg. K.352 will produce permanent cures in rabbits infected with *T. rhodesiense*. Longer courses of treatment with the same dose produce no toxic symptoms in normal rabbits.

TABLE VII

C. Tryparsamide. (1) Single Doses

Serial no. of rabbit	Initial dose (gm. per Kg.)	Later doses	Result of treatment
35	0·4	—	Accidentally killed 3 days later.
45	0·5	 0·8 (16)	Lesions not cleared. CURED. 10 months.
47	0·75	—	Slight loss in weight. CURED $10\frac{1}{2}$ months.
59	0·75	—	„ „ „ 10 „
60	0·75	—	„ „ „ 10 „
84	0·75	—	CURED. Still well 14 months.
34	0·8	—	„ 12 months.

Conclusion

0·75 gm. per Kg. tryparsamide can be administered with safety to rabbits infected with *T. rhodesiense* and will produce permanent cures.

TABLE VIII

C. Tryparsamide. (2) Serial Doses

Therapeutic Tests

Serial no. of rabbit	Number of doses (gm. per Kg.)	Length of course (days)	Result of treatment
105	3 × 0·25	6	CURED. Still well 8 months.
107	3 × 0·25	6	„ „ 8 „
108	6 × 0·25	16	„ „ 8 „
109	6 × 0·25	16	„ „ 8 „

Toxicity Tests

Serial no. of rabbit	Number of doses (gm. per Kg.)	Length of course (days)	Result of treatment
119	15 × 0·25	33	No toxic effects. Rabbit gained weight throughout.
120	15 × 0·25	33	„ „ „ „ „ „
118	11 × 0·4	24	„ „ „ „ „ „
121	11 × 0·4	24	„ „ „ „ „ „

N.B. Rabbits 119, 120 and 118 are still alive and healthy 6 months after last dose. Rabbit 121 killed itself 7 days after last dose by getting its neck caught in the cage.

Conclusion

Short courses of treatment with 3–6 doses of 0·25 gm. per Kg. tryparsamide will produce permanent cures in rabbits infected with *T. rhodesiense*. Longer courses of treatment with the same dose produce no toxic symptoms in normal rabbits.

DISCUSSION

The group of thioarsinites, which include the two compounds which are the subject of this communication, were originally chosen for study for a variety of reasons. Experience over a number of years had shown that pentavalent arsenicals were very prone to produce nervous symptoms in mice. It was to overcome this difficulty that attention was directed to the corresponding oxides, which, although they were thirty times more toxic than the parent acid, produced no nervous symptoms in mice when given in doses more than sufficient to clear the peripheral blood of trypanosomes. This is due, no doubt, to the relatively low dosage of combined arsenic.

The inherent disadvantages of arsenoxides are their weak acidic nature, often necessitating the use of caustic alkali for their solution, and the immediate manifestation of their toxic properties on injection. It was possible that these disadvantages could be overcome by condensation of the oxides with thiol compounds containing carboxyl groups. The thioarsinites so formed (usually crystalline substances which could be obtained pure) would then give neutral sodium salts.

A number of thioarsinites were examined (Cohen, King and Strangeways, 1931) and were found to be less toxic to mice than their content of arsenoxide would suggest, indicating that, when injected into the mammalian blood-stream as neutral sodium salts, they do persist as molecular units for a short time at least. They were also very effective in producing ' permanent cures ' in mice infected with *T. equiperdum*, as shown by no reappearance of trypanosomes in the blood over a period of 30 days. Although permanent in their effect the thioarsinites are, nevertheless, transitory in action, since they have little or no apparent influence on an infection given 24 hours after the drug.

The results obtained by Murgatroyd, Russell and Yorke (1934) on the trypanocidal titre of the serum of rabbits after the intravenous injection of an arseno-compound, a thioarsinite and an arsonic acid illustrate clearly the different behaviour of these three types of compound. A comparison of the effect of the thioarsinite and the arsonic acid, in our view supports the trial of thioarsinites as therapeutic agents. The sudden appearance in the blood-stream of a high concentration of trypanocidal agent brought about by the injection of a thioarsinite, followed by its rapid disappearance (by diffusion through the tissues as well as by excretion), may produce a condition more favourable to a complete eradication of parasites from the animal organism than that resulting

from the injection of an arsonic acid, when a relatively low, though longer persistant, concentration of the directly trypanocidal agent is produced.

The two thioarsinites, K.324 and K.352, were selected by preliminary sorting out on *T. equiperdum* infections in mice, as being superior to any of the other thioarsinites examined. In the foregoing account they have also been shown to have a high curative action in a single dose, which is only a fraction of the maximum tolerated dose, against *T. rhodesiense* and *T. brucei* in mice. On rabbits infected with *T. rhodesiense*, cures were effected by single intravenous doses of K.324 and K.352, but more effectively by a short course of 3–8 smaller doses. Normal rabbits on serial doses for a much longer period showed no ill effects and gained weight throughout. The fact that both substances contain naturally occurring thiol compounds may also be quoted in their favour.

It is of interest and significance to contrast the relative weights of arsenic necessary to cure *T. rhodesiense* infections in rabbits, when given in the form of K.324 or K.352, with the amount necessary in the case of a pentavalent arsenical such as tryparsamide. The results obtained with repeated injections of small doses of each drug have been used as a basis for the calculation. It was found that a total dose of 0·03 gm. per Kg. of K.324, given in 3 injections of 0·01 gm. per Kg., 0·04 gm. per Kg. of K.352, given in 4 injections of 0·01 gm. per Kg., and 0·75 gm. per Kg. of tryparsamide, given in 3 injections of 0·25 gm. per Kg., would effect permanent cures in the infected animals. The relative arsenic content of these doses is given in Table IX.

TABLE IX

Compound	Arsenic content per cent.	Curative dose (gm. per Kg.)	Arsenic content of curative dose	Relative arsenic content of curative dose
Tryparsamide $C_8H_{10}O_4N_2NaAs, 4H_2O$	20·4	0·75	0·153 gm.	100
K.324 $C_{13}H_{18}O_5N_3S_2As$	17·2	0·03	0·00516 gm.	3·4
K.352 $C_{28}H_{18}O_5N_3S_2As$	8·9	0·004	0·00356 gm.	2·3

It will be seen from the above Table that the amount of arsenic necessary to cure an infection of *T. rhodesiense* in a rabbit, when given in the form of K.324 or K.352, is only 2–3 per cent. of that needed when injected as tryparsamide. On general grounds it will be agreed that it is undesirable to introduce large amounts of arsenic into the mammalian organism, and, from this point of view alone, these thioarsinites have distinct advantages which merit further exploration.

In conclusion, therefore, the results obtained with the two thioarsinites K.324 and K.352, which are reported in the present communication, seem to

justify further investigation into the best methods for their administration to experimental animals, with a view to their therapeutic trial under natural conditions in the field.

SUMMARY

1. The two aromatic thioarsinites K.324 and K.352 are effective in curing *T. equiperdum*, *T. rhodesiense*, *T. brucei* and *T. gambiense* infections in mice in doses which are only a fraction of the maximum tolerated.

2. Neither compound has any effect on *T. congolense* infections in mice.

3. Rabbits infected with *T. rhodesiense* can be cured with single intravenous doses of both compounds, but more effectively by a short course of 3 to 8 smaller doses.

4. The relatively small amount of arsenic required to effect permanent cures in rabbits infected with *T. rhodesiense* when administered as the two thioarsinites, compared with that required when given as an arsonic acid such as tryparsamide, is discussed.

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REFERENCES

Browning, C. H., Cappell, D. F., and Gulbransen, R. (1934). Experimental infection with *Trypanosoma congolense* in mice : the effect of splenectomy. *Jl. Path. & Bact.*, **39**, 65.

Cohen, A., King, H., and Strangeways, W. I. (1931). Trypanocidal action and chemical constitution. X : Arylthioarsinites. *Jl. Chem. Soc.*, 3043.

——— ——— ——— (1932). Trypanocidal action and chemical constitution. XIII : Arylthioarsinites from cysteine and glutathione. *Ibid.*, 2505.

Murgatroyd, F., Russell, H., and Yorke, W. (1934). Studies in Chemotherapy. XI : The trypanocidal titre of serum of rabbits after the intravenous injection of various compounds of arsenic. *Ann. Trop. Med. & Parasitol.*, **28**, 227.

Pearce, L., and Brown, W. H. (1918). Experimental trypanosomiasis : its application in chemotherapeutic investigations. *Jl. Exper. Med.*, **28**, 109.

——— ——— (1919). The therapeutic action of *N*-phenylglycineamide-*p*-arsonic acid in experimental trypanosomiasis of rabbits. *Ibid.*, **30**, 455.

——— ——— (1921). Therapeutic action of *N*-phenylglycineamide-*p*-arsonic acid (tryparsamide) upon experimental infections of *Trypanosoma rhodesiense*. *Ibid.*, **33**, 193.

STUDIES ON BILHARZIA IN MAURITIUS*

II. THE RECOVERY OF ADULT *SCHISTOSOMA HAEMATOBIUM* AFTER DEVELOPMENT IN *BULINUS* (*PYRGOPHYSA*) *FORSKALI*

BY

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In a previous communication (Adams, 1934), experimental infestation of *Bulinus* (*Pyrgophysa*) *forskali* with miracidia hatched from ova obtained from local cases of vesical bilharzia was recorded. These observations have been repeatedly extended and confirmed at this laboratory during the last twelve months, and successive batches of the local variety of this particular species of snail have been infected in a similar manner with the greatest ease and regularity. No other snail of the many local species of fresh-water molluscs has been found experimentally to serve as a host for the parasite, and the writer is of the opinion that *B. forskali* represents the sole local intermediate host of *Schistosoma haematobium*. This belief is implemented by the fact that elsewhere in the Mascareigne group of islands, and in Madagascar, urinary bilharzia does not occur, although the fresh-water fauna of those territories is practically identical with that found in Mauritius, with the outstanding exception of *B. forskali* which occurs in this island alone.

A limited preliminary survey of the distribution of this species of snail in Mauritius has revealed its presence in all the main rivers and streams throughout the north-western area of the island—that area, in fact, from which most cases of urinary bilharzia are reported. Time and opportunity have not permitted of a full survey of other districts in the island, but occasional visits to rivers and streams where there appeared reasonably conclusive evidence that infestation with the disease had occurred in man resulted, in every instance, in the collection of specimens of this mollusc. There is little doubt that it is ubiquitous in its distribution throughout the running water of the island ; and it is hoped that in the future opportunity may present of demonstrating conclusively this fact.

PRELIMINARY ANIMAL INFECTION EXPERIMENTS

On obtaining numbers of experimentally infected *B. forskali*, sundry attempts were made to induce the bifid-tailed cercariae emerging therefrom to enter small animals, with the ultimate object of obtaining the adult male and female worms, and so of confirming the conclusions already reached as to the nature of the cercariae already obtained from these snails. The preliminary experiments were performed on mice and guinea-pigs ; three of each of these

* From the Bacteriological Laboratory, Medical and Health Department, Mauritius.

varieties of animal were taken, their abdomens were closely shaved, and on the shaved skin of each was placed about 20 c.cm. of water in which had rested for three or four hours a number of *B. forskali*, each observed to be emitting characteristic bifid-tailed cercariae. These snails had been infected *in vitro* some four to six weeks beforehand, and none had been found naturally infected after examination for some days before the experiment was begun. The time of application of the infecting fluid to each animal was 20 minutes in each case, and the application was made by introducing the fluid through the stem of an inverted funnel closely applied to the abdomen of the animal. Examination of the skins of the animals after the exposures in most cases showed that there was considerable reddening and frequently a minute rash-like appearance, while in the water remained numbers of free tails of the larvae and but very few intact cercariae. In no case were more than 250 larvae applied to any one animal, and in certain cases the number was very much less than this figure (50–100). After a period of three months the animals were all killed and dissected ; in no instance was there any indication of schistosomal infestation, and from no animal were any trematodes recovered.

The above experiment was repeated on a second similar series of animals ; in the mouths of a third series were inserted crushed infective snails, two to each animal ; while in a further series were inoculated peritoneally the crushed infective liver-glands of more snails, again two to each animal. After periods ranging from three to five months all these animals were killed and dissected. In none were schistosomes found at post-mortem. In one guinea-pig there was marked lobular cirrhosis of the liver; in another, which had received an intra-peritoneal inoculation, there was a sterile peritonitis with free pus in considerable quantity.

A total of 9 mice and 18 guinea-pigs were employed in the work described above ; and from none of these animals was there obtained any indication of successful parasitization.

The above facts require explanation. Estimations of the numbers of cercariae applied to each animal were made at the time by direct counting, where this was possible. In no instance were more than about 500 cercariae applied to any one animal, and in many cases the number was very considerably below this figure. In the light of further knowledge, the writer is of the opinion that a fear of over-infestation, with the consequent early deaths of infected animals due to portal obstruction by immature worms, had resulted in the application of insufficient infective larvae to each animal. This fear was not allayed until much valuable time and material had been consumed, and the experiment detailed later tends to show that the explanation is the correct one.

The literature on the subject of animal experimentation in *S. haematobium* researches is not very instructive in detail to those embarking on such work. The inference to be drawn from even a cursory study of the available work leaves one with the impression that over-infestation is the main obstacle to be

encountered in bringing such experiments to a satisfactory conclusion. It is, indeed, of some satisfaction to note that so eminent a worker as Leiper (1918) was delayed in a precisely similar manner during his well-known researches in Egypt. More recently a paper by Gordon, Davey, and Peaston (1934) has reached the writer's hands, unfortunately too late to avoid the initial pitfalls into which he had fallen ; Gordon *et al.* show that the number of cercariae of *S. haematobium* which reach maturity in the bodies of animals and which are recoverable at post-mortem after suitable time has elapsed is extraordinarily small in relation to the number to which the animal is exposed and which may be assumed to have penetrated. They apparently attribute this to immunity, or relative immunity, of the individual animals of various strains. In a paper otherwise replete with technical detail it seems unfortunate to the writer that they unaccountably omit clearly to state how they applied their infecting cercariae, and also their technique for enumeration of the very large numbers of larvae which they at times applied.

SUCCESSFUL ANIMAL INFESTATION

A clean laboratory-bred white mouse, kept under conditions in which there was no possibility of its becoming accidentally infected, was exposed to the attack of probably many hundred cercariae over a period of many hours. The animal was placed in a narrow glass tube in which it was obliged to ' stand ' in a semi-upright position. Water containing about two dozen *B. forskali*, each giving off freely characteristic furcocercous cercariae resulting from an experimental infestation, was added to the vessel until the mouse was half immersed. The animal was left in the water containing the living snails for two hours, and the operation was repeated daily for 12 days. This mouse, therefore, was exposed over a period of 24 hours to all the cercariae emerging in that time from about two dozen infective snails.

Almost precisely four calendar months later the mouse was found dead. A post-mortem examination was performed as soon as possible. Superficially little pathological change was observed in the abdominal or thoracic viscera. On careful dissection about 60 adult male and female schistosomes were recovered from the liver and portal vein, about 20 from the mesenteric vessels, and over 120 from the lungs. None were found in the pelvic vessels or bladder ; none in the spleen or kidneys.

The majority of the female worms were found *in copula*, and they were present in approximately equal proportions in both the liver and lungs, as well as in the mesenteric vessels. All worms recovered were alive on removal, but a number were lost or broken owing to difficulty experienced in freeing them from the vessels in which they lay. Males were present approximately in the ratio of twelve to one female. In the vessels at the roots of the lungs, in the immediate vicinity of the portal vein, and at the back of the mesentery in the larger vessels the worms were lying in particularly closely tangled masses.

Centrifugation of the saline solutions in which these various organs had been dissected resulted in the recovery of many characteristic terminal-spined eggs containing viable miracidia. These miracidia were readily induced to hatch out in clean water, and freely attacked and entered clean *B. forskali* with which they were placed in contact. It is hoped to repeat the cycle with these snails.

Fixation and staining of the adult worms showed them to conform to every major detail of the morphology of *S. haematobium*. The cuticle of the male, and the number. size and position of the testes were in conformity with the usual published descriptions of the species, while the position of the ovary in the female amply confirmed the identity. All the worms examined were sexually mature.

DISCUSSION

In considering the post-mortem findings in the case of this mouse, it is interesting to observe the preponderating number of adult sexually mature worms recovered from the pulmonary area. These worms were of both sexes, and with few exceptions the females were paired with males. This finding is in complete accord with that of Brumpt (1928), in which he records, of mice experimentally infected with the same species of schistosome, ' Les poumons renfermaient parfois des vers adultes et des oeufs,' while in some of his animals no worms were recovered at all from the intestine and bladder areas. In the abortive attempts to produce animal infection recorded above, it is well to remark that comparatively superficial examinations were made of the lungs in several instances ; it is thus possible that very light infestations confined to this area may have been overlooked, and the erroneous conclusion drawn that a complete lack of infestation had taken place.

In my possession are a number of shells of *Physopsis natalensis* kindly donated by Dr. Cawston of Durban. On studying these shells one is immediately struck by the very large size of the snail in comparison with that of *B. forskali*. This observation equally applies to *P. africana* and *B. contortus*, of which there are scale or life-size drawings available. The average dimensions of the shells of three *P. natalensis* are 14 mm. in length (apex to the lower end of the anterior canal) and 10 mm. in breadth (edge of outer lip to centre of lowest convolution on the opposite side). Those of 20 unselected *forskali*, representative of the snails used throughout the work described above, are 6·8 mm. in length and 3·8 mm. in breadth. It is thus evident that the bulk of a single *P. natalensis* is very much greater than that of a single specimen of the local variety of *B. forskali*, and it is not unreasonable to deduce that the number of cercariae which an infected *P. natalensis* may emit may be very much greater than that given off by a similarly infected *B. forskali*. In my opinion this may well explain the fact that a single crushed *natalensis* placed under the tongue of a guinea-pig will frequently produce infection in the animal, while repeatedly this and similar procedures have failed in my hands using one, two or even three infected local

B. forskali. Where *B. forskali* are found in the local canals and streams they are found usually in very large numbers. A trained ' boy ' can collect many hundreds of this species in an hour or so, as where a single snail is found very large numbers are in the immediate vicinity. It has been shown above that numbers of infective molluscs over a period will cause infestation where a single snail, or two or three snails, will fail to do so. In nature, in Mauritius, the high concentration of numbers occurs owing to the fecundity and tendency of the snails to congregate in suitable environmental conditions, and thus the necessary degree of concentration of infective larvae is provided for, assuming that man has some natural resistance to mild degrees of infestation, as would appear to be the case with mice and guinea-pigs.

I am very greatly indebted to the Medical Research Institute of South Africa for the gift of a number of white mice, which have founded a nucleus of stock available for experiments at this laboratory. To my assistant Mr. Lewis Webb I am very grateful for much painstaking and devoted assistance and intelligent co-operation.

SUMMARY

1. The fact that *Bulinus* (*Pyrgophysa*) *forskali* is readily infected with miracidia obtained from local cases of *Schistosoma haematobium* infection in man has been repeatedly confirmed.

2. No other snail host has been incriminated as a vector of this parasite in Mauritius, and reasons are adduced against there being a second molluscan intermediary.

3. A number of unsuccessful experiments were performed on mice and guinea-pigs in an attempt to obtain adult worms from the furcocercous cercariae emerging from experimentally infected *B. forskali*.

4. The unsuccessful nature of these experiments is accounted for on the grounds that insufficient cercariae were applied to each animal.

5. A mouse was eventually heavily infested with mature adult worms by long exposure to massive numbers of cercariae.

6. From this animal were recovered about 200 schistosomes characteristic of *S. haematobium*. Characteristic terminal-spined ova were found in the tissues from which the worms had been removed. From these ova emerged viable miracidia which readily attacked and penetrated ' clean ' *B. forskali*.

7. The schistosomes were found chiefly in the lungs ; the liver was second in point of numbers, while few were recovered from the mesenteric vessels. Males were present in the ratio of about twelve to one female.

8. The detailed morphology of the worms of both sexes was that of *S. haematobium*, and thus there is conclusive evidence that *Bulinus* (*Pyrgophysa*) *forskali* acts as a molluscan host of this parasite.

9. Attention is drawn to the fact that the local variety of *B. forskali* is a comparatively small snail in relation to the other known molluscan hosts of

S. haematobium. Numbers and concentration of the snail locally would surmount the difficulty of massive infestation if this is necessary in man as in small laboratory animals, assuming that snails are capable of a degree of infestation proportional to their respective sizes.

REFERENCES

ADAMS, A. R. D. (1934). Studies on bilharzia in Mauritius. I: The experimental infection of *Bulinus (Pyrgophysa) forskali* with *Schistosoma haematobium*. *Ann. Trop. Med. & Parasitol.*, **28**, 195.

BRUMPT, E. (1928). Réalisation expérimentale du cycle complet du *Schistosoma haematobium*. *Ann. Parasitol.*, **6**, 440.

GORDON, R. M., DAVEY, T. H., and PEASTON, H. (1934). The transmission of human bilharziasis in Sierra Leone, with an account of the life-cycle of the schistosomes concerned, *S. mansoni* and *S. haematobium*. *Ann. Trop. Med. & Parasitol.*, **28**, 323.

LEIPER, R. T. (1918). Report on the results of the Bilharzia Mission in Egypt, 1915. V.: Adults and ova. *Jl. Roy. Army Med. Corps*, **30**, 235.

SCREENCLOTH FOR HOUSES IN THE TROPICS

BY

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In referring to malaria and to the success of his original Roman Compagna experiment, in which screening of the doors and windows of the experimental house by wire gauze was the chief means of protection used, Manson wrote : ' The question of expense cannot for a moment be entertained in discussing the means for protection. One life saved, one invaliding obviated, would, even in a pecuniary sense, pay for all the wire gauze and mosquito netting requisite to protect every European house in West Africa.'

There are reasons for believing that in the near future the use of wire mosquito-netting for the purpose of screening houses as a protection against malaria is likely to be considerably extended in tropical countries.

In the past, one of the most serious objections to the screening of houses in such climates as those of West Africa and other similar parts of the tropics has been the degree of stuffiness, due to interference with ventilation, which resulted from equipping buildings with small-mesh netting. Nowadays, the introduction of electric supplies in many places in the tropics has removed much of the weight of this objection, because fans can be installed without much expense, thereby very greatly improving the ventilation and comfort inside a mosquito-proofed room or house.

Screening has hitherto been applied more particularly to barracks, hospitals, schools and various institutions where large numbers of people require protection; but it seems reasonable to suppose that, taking advantage of modern developments, many more people will be desirous of trying the effect of screening their private dwellings than was formerly the case.

Another obstacle to the popularization of screening has been the difficulty which the ordinary individual has hitherto found in deciding on what mesh of wire screencloth he should order for his buildings with the certainty that it would suffice to exclude mosquitoes. This matter has been carefully investigated by MacArthur, who came to the conclusion, as a result of his experiments with *Aëdes* (*Stegomyia*) *fasciata*, that a wire screencloth having 14 meshes to the linear inch and composed of strands of wire of no. 30 Imperial Standard Wire Gauge was entirely adequate.

For the purpose of screening water-containers such as butts and cisterns, MacArthur recommended screencloth of 18 meshes to the linear inch, composed of strands of wire of no. 30 Imperial Standard Wire Gauge. This smaller mesh was recommended on account of the fact that the bodies of newly hatched *Aëdes* are more pliable and might be able to push through the wider mesh. The amount of light admitted through the mesh is important in house screening but

not in the case of water barrels and other containers in which mosquitoes may breed ; a stronger and more durable wire such as 28 Imperial Standard Wire Gauge could therefore be used for covering such containers. It has become clear, as a result of these experiments, that, so far as the protection of dwelling-rooms against mosquitoes is concerned, a multiplicity of meshes and of thicknesses of wire can be avoided. Thus for all practical purposes only two sizes of mesh and wire need be considered in making an order for screencloth. These are :—

(*a*) For the screening of buildings, a 14-mesh screencloth made of wire of 30 Imperial Standard Wire Gauge.

(*b*) For the screening of water-containers which breed mosquitoes, an 18-mesh screencloth made of wire of 30 or 28 Imperial Standard Wire Gauge.

Cost. While agreeing in principle with Manson's dictum, and desirous of acting upon it, most local authorities have found that the question of cost has invariably to be considered, and individuals are equally affected by it. In connection with this, it should be observed that the price has hitherto been kept up largely on account of the irregular orders for small quantities of screencloth, specifying many different meshes and wire thicknesses. Provided the demands upon the manufacturer could be limited to a few types of screencloth, a great economy in manufacture would inevitably result. So long as manufacturers have to set up and adjust machines to produce a large variety of sizes of mesh using different thicknesses of wire, the cost is bound to remain high, since one of the most important items of cost in making screencloth is the setting up of the machines at the beginning. It takes as much time, labour and expense to set up and adjust a machine to fulfil an order for a few yards of wire screencloth as it does to set up the same machine to fulfil an order for many hundreds of yards. Thus we see that, if we can confine our requisitions of wire screencloth to one or two types, the result will be that the manufacturers will produce these in bulk and will accumulate a large stock of the manufactured article. They will therefore be able to reduce very materially the cost of screencloth for houses in the tropics, and, further, they will be able to fulfil orders expeditiously from stock.

Metals. Should many different kinds of metal be specified in ordering small quantities of screencloth, similar difficulties of initial cost arise for the manufacturer. Much progress will be made if we decide not only upon the most practical size of mesh and the most suitable gauge of wire, but also on one kind of metal which has been proved to give useful service in the tropics, and which is at the same time cheap enough to be within the reach of anyone who desires to protect his house.

In Sierra Leone an experiment was carried out with a screencloth constructed of wire of a metal known by the name Barronia which is an entirely British product. This screencloth was fixed in the windows of an animal-house in 1928, and was left in position for well over two years. During this time it was exposed

not only to the usual effects of heat and moisture, but also to the unusual effects of very severe conditions of weathering from the direct impact of wind and rain during the tornado seasons ; there were no shutters or glass windows to protect the wire in any way. Samples of this exposed wire were removed at the end of the trial period for examination ; the specimens showed some discolouration but had not suffered any further damage, and the wire showed no signs of wastage or corrosion. Barronia metal wire proved itself therefore a particularly good wire in the exacting conditions of the tropics as represented by the climate of Sierra Leone ; it is definitely superior to phosphor-bronze and copper, and it compares very favourably as regards cost with Monel metal wire which is also recommended as eminently suitable for resisting corrosion at sea-level in the tropics.

The difference in price will be realized from the following extract from a recent price list of one British manufacturer* of woven metal.

Mesh	Gauge of wire	Material	Price per 100 sq. ft. of screencloth
			s. d.
14 × 14	30	Barronia metal	45 8
		Monel metal	65 6
18 × 18	28	Barronia metal	80 0
		Monel metal	116 9

It is important to note that mosquito-netting made of Barronia wire does not require painting, as the slight film which forms on the wire, due to weathering, acts as a protective covering to resist corrosion.

*N. Greening & Sons, Ltd., Britannia Works, Warrington.

REFERENCES

MacArthur, W. P. (1923). Mosquito netting. *Jl. Roy. Army Med. Corps*, **40**, 1.
Manson, P. (1900). Experimental proof of the mosquito theory. *Brit. Med. Jl.*, **2**, 949.

STUDIES IN CHEMOTHERAPY*

XII.—THE DIFFUSIBILITY OF THE AROMATIC ARSENICALS INTO ERYTHROCYTES AND THE ACTION OF THE LATTER ON THE PENTAVALENT ARSENICALS

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In a recent paper (1934), we recorded the trypanocidal titre of the serum of rabbits at various intervals after the intravenous injection of certain aromatic compounds of arsenic. It was found :—

1. That the effect of injection of an arsenobenzol compound (N.A.B.) or of an aromatic trivalent arsenical compound (reduced tryparsamide thioglycollate†) is to confer immediately upon the serum an enormously high trypanocidal titre ; this titre, which is proportional to the dose given, immediately falls—quickly at first and more slowly later—until it ultimately returns to zero. The fall in the case of reduced tryparsamide thioglycollate is much more rapid than in that of novarsenobillon.
2. That the immediate effect of injection of an aromatic pentavalent arsenical compound (tryparsamide) is to confer but a relatively low trypanocidal titre upon the serum ; instead of falling, however, as happens with the other two drugs, the titre steadily rises and does not attain to its maximum for some time after injection. The titre reached is, moreover, in no way comparable with the enormous titres obtained with novarsenobillon and reduced tryparsamide thioglycollate.
3. That, whereas in the case of the arsenobenzol compound the trypanodical titre exhibited by the serum 2½ minutes after intravenous injection approximated fairly closely to the calculated value, in the case of the other two compounds the titres observed were only small fractions of the calculated values.

These observations naturally raise certain important questions, the solutions of which may well assist materially in the understanding of the mechanism of therapeutic processes. In view of the fact that we have shown (1930) that arsenobenzol and aromatic trivalent compounds exhibit an enormous trypanocidal power *in vitro*, it is only to be expected that the immediate effect of their introduction into the blood stream will be to confer a high trypanocidal titre upon the serum. But why in the case of N.A.B. does the titre observed 2½ minutes after injection approximate to the calculated titre, whilst in the case of reduced tryparsamide thioglycollate it is only a small fraction of the calculated value ; and why during the hours which follow the injection does the titre

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†Disodium di (carboxymethyl) 4-glycineamido-phenyl thioarsinite.

fall so much more rapidly in the case of reduced tryparsamide than in that of novarsenobillon ? Again, since we have shown that aromatic pentavalent arsenicals exert but little trypanocidal power *in vitro*, it is not surprising that the immediate effect of the introduction of tryparsamide into the blood stream is to confer but a relatively low trypanocidal titre upon the serum. Why, however, should the titre observed 2½ minutes after intravenous injection of tryparsamide prove to be only a small fraction of the calculated value, and why should the titre rise during the next 6 hours instead of falling as in the case of the other compounds ?

One explanation for the rapid fall in the trypanocidal titre of the serum, which is observed especially in the case of the aromatic trivalent compounds and, to a less extent, in that of the arsenobenzol compounds, is that these substances are rapidly excreted from the body ; we intend to discuss this matter in a later paper. Such a hypothesis cannot, however, suffice to explain the fact that 2½ minutes after intravenous injection of reduced tryparsamide, or of tryparsamide itself, but a small fraction of the arsenical injected can be found in the serum. The only adequate explanations of this phenomenon would seem to be either that the drugs are at once rendered partly inert when injected into the blood stream, or that they are removed from the *liquor sanguinis* by the body cells with amazing speed. Very little, if anything, appears to be known concerning the diffusibility of the aromatic arsenicals, and yet it is obvious that it is upon this character that the various phenomena mentioned above may, in no small measure, depend. The subject seemed to us, therefore, to be worthy of investigation.

In casting round for a technique whereby it would be possible to examine the diffusibility of the drugs in question, it occurred to us that the red blood corpuscle would afford a suitable experimental medium. It has no nucleus and is of more homogeneous structure than the true body cells, it is easily handled, and, as it is a constituent of the circulating blood, it would at once be brought into intimate contact with any substance introduced into the circulation.

DIFFUSIBILTY OF REDUCED TRYPARSAMIDE INTO ERYTHROCYTES

A number of experiments was performed in which rabbit red cells were suspended in various solutions of reduced tryparsamide ; after being kept at 37° C. for various periods, the red cells were separated from the drug solution, washed thoroughly in iced saline, and their content of drug determined. The following is a typical experiment :—

A Ringer-glucose solution containing 1 : 25,000 reduced tryparsamide was prepared. The trypanocidal titres of this solution, freshly-made and after keeping at 37° C. for 6 hours, were determined in the usual way* (Table I, Specimens 1 and 2).

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*The procedure adopted for determining the trypanocidal titre was that described in our previous papers, i.e., the incubation at 37° C. for 24 hours of trypanosomes in nutrient medium containing various concentrations of the specimens under examination.

To 1·8 c.cm. of this freshly-made solution was added 1·8 c.cm. of rabbit washed red corpuscles and the mixture left in a water-bath at 37° C. for 3 hours. It was then divided into two equal parts A and B, which were treated as follows :—

Portion A.—The red cells (0·9 c.cm.) were separated from the Ringer-glucose-drug solution by centrifugation, washed once in 9 times their volume of iced saline (8·1 c.cm.) and then laked by the addition of 6·0 c.cm. of distilled water. The laked red cells were allowed to stand at 37° C. for 30 minutes, after which 0·3 c.cm. of 20 per cent. NaCl solution was added, and the stroma removed by high speed centrifugation. The trypanocidal titre of the laked red cells was then determined (Table I, Specimen 3).

Portion B.—The red cells (0·9 c.cm.) were separated from the Ringer-glucose-drug solution (' Extracted ' solution) by means of the centrifuge and washed rapidly three times in 8·1 c.cm. of iced saline. To the washed red cells was then added an equal volume (0·9 c.cm.) of normal Ringer-glucose solution (' Extracting ' solution) and the mixture allowed to stand for 3 hours at 37° C. ; it was then centrifuged, the ' Extracting ' solution removed, and the red cell deposit washed once again in 8·1 c.cm. of iced saline. To the washed red cells (0·9 c.cm.) was then added 6·0 c.cm. of distilled water and the laked red cells were allowed to stand at 37° C. for 30 minutes, after which 0·3 c.cm. of 20 per cent. NaCl solution was added, and the stroma removed by high speed centrifugation. The trypanocidal titres of the following specimens were then determined (Table I, Specimens 4–10).

4. ' Extracted ' Ringer-glucose-drug solution
5. Saline used for 1st washing.
6. „ „ „ 2nd „
7. „ „ „ 3rd „
8. ' Extracting ' normal Ringer-glucose solution.
9. Saline used for washing extracted corpuscles before laking.
10. Laked red cells after extracting and washing.

This experiment shows clearly that, when normal red cells are suspended in Ringer-glucose solution containing 1 : 25,000 reduced tryparsamide for 3 hours at 37° C., a certain amount of the drug diffuses into the red cells. The first portion of the experiment, in which the red cells, after remaining in contact with the drug solution for 3 hours, were washed once in iced saline and then laked with distilled water, shows that about one-eighth of the total amount of drug had passed into the red cells (Table I, Specimen 3). This finding is confirmed by the more elaborate second portion of the experiment. In this part of the experiment the red cells, which had stood for 3 hours at 37° C. in Ringer-glucose-drug solution, were washed thrice in 9 times their volume of iced saline. The trypanocidal titres of the saline washings were ascertained ; that of the first washing exhibited a considerable trypanocidal power, that of the second washing only a slight trypanocidal power, and that of the third washing none. Examination of these figures suggests that little, if any, of the drug diffused out of the red cells into the saline washings under the conditions of the experiment, i.e., within 2 or 3 minutes at 0° C. The relatively high titre of the saline of the first washing can, of course, be explained by the fact that a small portion of the Ringer-glucose-drug solution was of necessity left behind with the red cell deposit after centrifugation.

When these thoroughly washed red cells were allowed to stand in an equal volume of normal Ringer-glucose solution for 3 hours at 37° C., a certain

TABLE I

Showing the diffusibility of reduced tryparsamide from Ringer-glucose solution into rabbit red blood corpuscles

	No. of specimen	Specimen	No. of times specimen diluted	Concentration of drug	No. of living trypanosomes per 256 squares of the haemocytometer scale	
					Start	After 24 hours at 37° C.
	1	Original solution (1 : 25,000) of reduced tryparsamide in Ringer-glucose, freshly-made	4,096 8,192 16,384	102·4 mill. 204·8 „ 409·6 „	88	0 6 64
	2	Original solution (1 : 25,000) of reduced tryparsamide in Ringer-glucose, after 6 hours at 37° C.	2,048 4,096 8,192 16,384	51·2 „ 102·4 „ 204·8 „ 409·6 „		0 0 27 82
Portion A	3	Laked erythrocytes not previously extracted	256* 512 1,024 2,048			0 0 30 64
Portion B	4	'Extracted' Ringer-glucose-drug solution	4,096 8,192 16,384			0 58 72
	5	Saline of 1st washing	32 64 128			0 7 68
	6	Saline of 2nd washing	4 8			0 42
	7	Saline of 3rd washing	2 4			46 84
	8	'Extracting' normal Ringer-glucose solution	256 512 1,024			0 31 84
	9	Saline used for washing extracted corpuscles before laking	4 8 16			0 33 72
	10	Laked erythrocytes after extracting and washing	128* 256 512			0 60 76

*These figures represent multiples of the volume of red cells before laking

amount of drug was found to have passed from the washed cells into the Ringer-glucose solution (Table I, Specimen 8). The ' Extracted ' red cells were then again washed in 9 times their volume of iced saline, and subsequently laked by the addition of 7 times their volume of distilled water. The trypanocidal power exhibited by the solution of laked red cells (Table I, Specimen 10) is further evidence that the red cells contained a definite amount of drug.

The general conclusions which can be drawn from this experiment appear to be as follows :—

1. When red cells are suspended in an equal volume of Ringer-glucose solution containing 1 : 25,000 reduced tryparsamide for 3 hours at 37° C., a definite quantity of the drug amounting to approximately one-eighth that present in the Ringer-glucose solution diffuses into the red cells.

2. When the washed drug-laden red cells are suspended in normal Ringer-glucose solution for 3 hours at 37° C., a certain proportion of the drug they contain diffuses into the Ringer-glucose solution.

3. When such extracted red cells are washed and laked, the presence of drug can still be demonstrated in the laked solution.

4. At 0° C. the rate of diffusion of the drug out of red cells appears to be very slow.

Having by this means succeeded in demonstrating that reduced tryparsamide diffuses readily into and out of red cells at 37° C., we next proceeded to investigate the influence of such factors as time, and concentration of drug, upon the reaction. With these objects in view, we performed a number of experiments in which two concentrations of reduced tryparsamide in Ringer-glucose solution were used. The first was that employed in the previous experiment, viz., 1 : 25,000, and the second was sixteen times as great. In order to ascertain the effect of the time factor, observations were made after the red cells had been in contact with Ringer-glucose-drug solution for 15 minutes, 3 hours and 24 hours, respectively. Otherwise the conditions of experiment were exactly similar to those of previous experiments.

The points which emerge from experiments of this nature, the results of which are summarized in Table II, are :—

1. Column I shows that the trypanocidal titres of the Ringer-glucose-drug solutions fall but slightly during 24 hours at 37° C., in the absence of red cells.

2. Column II shows that in the case of both solutions, the amount of drug absorbed by the red cells within 24 hours is about twice as much as that absorbed within 15 minutes. The amount of drug absorbed from the stronger solution is roughly about 8 times as much as that absorbed from the weaker solution.

3. Column IV shows that the amount of drug 'extracted' from the red cells, which had been in contact with the drug solution for 24 hours, is much less than that ' extracted ' from the red cells which had been exposed to the drug solution for only 15 minutes. On the contrary, the amount of drug found in the laked

TABLE II

Showing the influence of concentration of drug, and time, on the diffusibility of reduced tryparsamide from Ringer-glucose solution into red corpuscles

Concentration of reduced tryparsamide in Ringer-glucose solution	Length of time Ringer-glucose-drug solution kept at 37° C. either alone or after addition of red cells	Trypanocidal titres of: Ringer-glucose-drug solution without addition of red cells Col. I	Laked drug-laden red cells not previously extracted by normal Ringer-glucose solution Col. II	'Extracted' Ringer-glucose-drug solution after removal of red cells Col. III	Normal Ringer-glucose solution used for extracting washed drug-laden red cells Col. IV	Laked drug-laden red cells previously extracted by normal Ringer-glucose solution Col. V
1 : 25,000	0 minutes	4,000–8,000				
	15 ,,	4,000–8,000	512–1,024	4,000	512–1,024	128
	3 hours		512–1,024	4,000	256–512	128–256
	6 ,,	4,000–8,000				
	24 ,,	4,000	1,024–2,048	2,000	128	1,024
1 : 1,562·5	0 minutes	65,500–131,000				
	15 ,,	65,500–131,000	4,000–8,000	65,500–131,000	2,000–4,000	2,000
	3 hours		4,000–8,000	65,500	2,000	2,000
	6 ,,	65,500–131,000				
	24 ,,	65,500	8,000–16,000	32,500	2,000	8,000

TABLE III

Showing the influence of concentration of drug, and time, on the diffusibility of reduced tryparsamide from 'nutrient medium' into red corpuscles

Concentration of reduced tryparsamide in nutrient medium	Length of time nutrient-medium-drug solution kept at 37° C. either alone or after the addition of red cells	Trypanocidal titres of: Nutrient-medium-drug solution without addition of red cells Col. I	Laked drug-laden red cells not previously extracted by normal nutrient medium Col. II	'Extracted' nutrient-medium-drug solution after removal of red cells Col. III	Normal nutrient medium used for extracting washed drug-laden red cells Col. IV	Laked drug-laden red cells previously extracted by normal nutrient medium Col. V
1 : 25,000	0 minutes	4,000–8,000				
	15 ,,		512–1,024	2,000–4,000	512–1,024	128
	3 hours	2,000	512–1,024	2,000–4,000	256–512	128–256
	6 ,,	2,000				
	24 ,,	256	1,000–2,000	1,000–2,000	128	1,000
1 : 1,562·5	0 minutes	65,500–131,000				
	15 ,,		4,000–8,000	65,000–131,000	2,000	2,000
	3 hours	65,500–131,000	4,000–8,000	65,500	2,000	2,000
	6 ,,	65,500–131,000				
	24 ,,	65,500	8,000–16,000	32,500	2,000	8,000

solution of the former red cells is much greater than that found in the laked solution of the latter (Column V).

These observations seem to allow of the following conclusions :—

1. The trypanocidal titres of solutions of reduced tryparsamide in Ringer-glucose remain almost unchanged during a period of 24 hours at 37° C.

2. The amount of drug absorbed by red cells varies with the strength of the solution and with the length of their sojourn in the solution, but the rate of diffusion is exceedingly great, as is shown by the fact that within 15 minutes the amount absorbed is about half as much as is absorbed within 24 hours. The fact that a much smaller proportion of the absorbed drug diffuses into normal Ringer-glucose solution from red cells which have stood in the drug solution for 24 hours than from those which have been in the drug solution for only 15 minutes, seems capable of explanation on the assumption that in the former instance the drug is more firmly bound to the substance of the red cells than in the latter case. Although this binding is sufficiently close to inhibit the diffusion out of the red cell into Ringer-glucose solution, it is not sufficiently firm to mask the trypanocidal action of the drug when the ' Extracted ' red cells have been laked subsequently with distilled water.

We next decided to ascertain whether the presence of serum in the solution in which the drug was dissolved exerted any influence on these various reactions. Accordingly, experiments were performed in all respects similar to those already described, except that the reduced tryparsamide was dissolved in ' nutrient medium ' (equal parts of rabbit's serum heated to 64° C. for ½ an hour and Ringer-glucose solution) instead of in Ringer-glucose solution alone. The results of these experiments are summarized in Table III.

With one striking exception, the results obtained in these experiments were identical with those obtained in the previous experiments. The drug diffused into and out of the red cells in precisely the same manner as when it was dissolved in Ringer-glucose solution. The exception referred to relates to the results shown in Column I of the Table. Here it is seen that, whereas the titre of the freshly-made weak solution of drug in 'medium' was 4,000–8,000, that of the same solution which had been kept at 37° C. for 24 hours was only 256. A similar striking fall in titre was not seen in the case of the strong solution of drug which had been kept at 37° C. for 24 hours. For some reason or other, therefore, the weak solution (1 : 25,000) of reduced tryparsamide in ' medium ' rapidly loses its trypanocidal power when it is stored at 37° C. This loss is not observed with the weak solution of reduced tryparsamide in Ringer-glucose, nor is it seen in solutions sixteen times as strong, whether the solvent be ' medium ' or Ringer-glucose. Column III discloses a very interesting phenomenon. In the case of the weak solution of drug, it is seen that the trypanocidal titre of the drug-containing-medium was only slightly less when an equal volume of red cells had been in contact with it for 24 hours than it was when the red cells had been in contact with it for only 15 minutes. In fact, the addition of an equal volume of red cells

to ' medium ' containing 1 : 25,000 reduced tryparsamide prevented the fall in trypanocidal titre which occurs when the drug-containing-medium is stored in the absence of red cells for 24 hours at 37° C.

The explanation of these phenomena is by no means clear ; possibly serum proteins have the power of anchoring minute quantities of the drug, thus inhibiting the trypanocidal activity of the latter. Such a hypothesis would explain the pronounced fall in trypanocidal titre noticed when a weak solution of drug in ' medium ' is kept at 37° C. for 24 hours, and the absence of such a fall in the case of the strong solution of drug in ' medium,' where presumably there is a relatively great excess of drug, and in those of both weak and strong Ringer-glucose solutions in which there is no protein present. It is difficult, however, on such a hypothesis to explain why the presence of red blood cells prevents the fall in trypanocidal titre of the weak solution of drug in ' medium ' which is normally observed when the latter is kept at 37° C. for 24 hours.

DIFFUSIBILTY OF TRYPARSAMIDE INTO ERYTHROCYTES

Similar experiments on the diffusibility into red blood corpuscles of the aromatic pentavalent arsenical compound, tryparsamide, produced results which made it immediately obvious that some factor was operating which completely obscured the main issue. The following is a typical experiment.

A Ringer-glucose solution containing 1 : 200 tryparsamide was prepared. The trypanocidal titres of this solution, freshly-made, and after keeping at 37° C. for 2 hours, were determined in the usual way (Table IV, Specimens 1 and 2). To 0·9 c.cm. of this solution was added 0·9 c.cm. of rabbit washed red corpuscles and the mixture left in the water-bath at 37° C. for 2 hours. The red cells (0·9 c.cm.) were then separated from the Ringer-glucose-drug solution (' Extracted ' solution) by means of the centrifuge and washed rapidly three times in 8·1 c.cm. of iced saline. To the washed red cells was then added an equal volume (0·9 c.cm.) of normal Ringer-glucose solution (' Extracting ' solution) and the mixture allowed to stand for 2 hours at 37° C. ; it was then centrifuged, the ' Extracting ' solution removed, and the red cell deposit washed once again in 8·1 c.cm. of iced saline. To the washed red cells (0·9 c.cm.) was then added 6·0 c.cm. of distilled water, and the laked red cells were allowed to stand at 37° C. for 30 minutes, after which 0·3 c.cm. of 20 per cent. NaCl solution was added, and the stroma removed by high speed centrifugation. The trypanocidal titres of the following specimens were then determined (Table IV, Specimens 3–9).

3. ' Extracted ' Ringer-glucose-drug solution.
4. Saline used for 1st washing.
5. ,, ,, ,, 2nd ,,
6. ,, ,, ,, 3rd ,,
7. ' Extracting ' normal Ringer-glucose solution.
8. Saline used for washing extracted corpuscles before laking.
9. Laked red cells after extracting and washing.

The results which are set out in Table IV show that Ringer-glucose solution containing 1 : 200 tryparsamide (Specimen 1) suffices to destroy trypanosomes at 37° C. within 24 hours when diluted four times. (The trypanocidal titre of tryparsamide is thus approximately 800.) When, however, an equal volume of red corpuscles was allowed to stand in the 1 : 200 solution of tryparsamide

for 2 hours at 37° C., it was found that the trypanocidal power of the solution, instead of being reduced somewhat, as in the case of the corresponding trivalent compound, was increased no less than 16 times, i.e., from 4 to 64 (Specimen 3). As in experiments with reduced tryparsamide, there was evidently no appreciable diffusion of drug from the loaded red cells into the saline at low

TABLE IV

Experiment on the diffusibility of tryparsamide from Ringer-glucose solution into rabbit red blood corpuscles

No. of specimen	Specimen	No. of times specimen diluted	Concentration of drug	No. of living trypanosomes per 256 squares of the haemocytometer scale: Start	No. of living trypanosomes per 256 squares of the haemocytometer scale: After 24 hours at 37° C.
1	Original solution (1 : 200) of tryparsamide in Ringer-glucose, freshly-made	2 4 8 16	1 : 400 800 1,600 3,200	90	0 0 60 74
2	Original solution (1 : 200) of tryparsamide in Ringer-glucose, kept 2 hours at 37° C.	2 4 8 16	1 : 400 800 1,600 3,200	90	0 0 56 84
3	'Extracted' Ringer-glucose-drug solution	16 32 64 128 256		90	0 0 0 45 80
4	Saline of 1st washing	2 4 8		90	1 82 72
5	Saline of 2nd washing	2 4		90	80 84
6	Saline of 3rd washing	2 4		90	66 88
7	'Extracting' normal Ringer-glucose solution	64 128 256 512		90	0 0 50 80
8	Saline used for washing extracted corpuscles before laking	2 4 8		90	0 6 72
9	Laked erythrocytes after extracting and washing	64 128 256 512		90	0 0 14 80

temperatures within a few minutes. The trypanocidal power of the normal Ringer-glucose solution used for extracting the washed drug-laden corpuscles was, moreover, even greater than that of the ' Extracted ' Ringer-glucose drug solution, i.e., 128 (Specimen 7) as compared with 64 ; and that of the laked corpuscles after extraction for 2 hours in normal Ringer-glucose solution was nearly 256 (Specimen 9).

These results seem to warrant two inferences, viz. : (1) that tryparsamide diffuses into red blood corpuscles to some extent, and (2) that part of the tryparsamide which diffuses into the red corpuscles is changed inside the corpuscles to some substance which is much more actively trypanocidal than tryparsamide itself.

CAPACITY OF ERYTHROCYTES TO ACTIVATE TRYPARSAMIDE

The increase in trypanocidal activity evidently varies to some extent with the length of time the tryparsamide is left in contact with the red blood cells, and this would seem to be the explanation of the fact that the trypanocidal titre of the ' Extracted ' Ringer-glucose-drug solution (Specimen 3) instead of being greater than that of the ' Extracting ' normal Ringer-glucose solution (Specimen 7) or of the laked erythrocytes (Specimen 9) is only half as great as either of them. In Specimen 3 the tryparsamide had been in contact with the red cells for only 2 hours, whereas in Specimen 9 it had been in contact with them for 4 hours.

Later experiments showed that the trypanocidal power of tryparsamide could also be increased by incubation at 37° C. in a solution obtained by laking red blood corpuscles. A solution was obtained by laking one volume of red blood cells with 7 volumes of distilled water, the stroma being removed by high speed centrifugation after the addition of sufficient sodium chloride to render the solution isotonic. For convenience this solution might be regarded as a 12·5 per cent. solution of red blood cells. Equal volumes of this solution and of a 1 per cent. solution of tryparsamide in Ringer-glucose solution were mixed and allowed to stand at 37° C. for 2 hours. The resulting mixture consisted, therefore, of 0·5 per cent. solution of tryparsamide in a 6·25 per cent. solution of laked red corpuscles. The trypanocidal titre of the mixture was then determined in the usual way. It was found to be about 1,000 as compared with 4 for a 0·5 per cent. solution of tryparsamide in Ringer-glucose solution. It follows, therefore, that a solution of red blood cells is capable of greatly increasing the trypanocidal power of tryparsamide.

Experiments were next undertaken with the object of ascertaining the effect of the following factors on the reaction, viz. : (*a*) duration of exposure of tryparsamide to red cell solution, (*b*) concentration of tryparsamide and (*c*) concentration of red cell solution. With this in view, 1 : 50, 1 : 200 and 1 : 800 solutions of tryparsamide in Ringer-glucose, and 25 per cent., 6·25 per cent. and 1·6 per cent. solutions of normal rabbit red blood cells were made. Equal volumes of these

various tryparsamide solutions were added to equal volumes of the red cell solutions, and the mixtures incubated at 37° C. for periods varying from 30 minutes to 6 hours. The trypanocidal titres of the incubated mixtures were then determined. The results, which are set forth in Table V, show that the titre

TABLE V

Showing the capacity of red cell solutions to activate tryparsamide under various conditions

Concentration of tryparsamide	Concentration of laked red cell solution (percentage)	Length of incubation at 37° C. of tryparsamide and red cell solution	Trypanocidal titre
1 : 100	3·1	2½ hrs.	1,024
1 : 400	3·1	2½ ,,	256
1 : 1,600	3·1	2½ ,,	32
1 : 400	12·5	2½ ,,	2,048
1 : 400	3·1	2½ ,,	256
1 : 400	0·8	2½ ,,	32
1 : 400	3·1	30 mins.	64
1 : 400	3·1	2½ hrs.	256
1 : 400	3·1	6 ,,	512
1 : 100	12·5	6 ,,	16,384
1 : 100	nutrient medium (Control)	6 ,,	10

obtained varied directly with the concentration of tryparsamide solution, with that of the red cell solution and with the duration of their contact at 37° C. When the concentration of tryparsamide was 1 : 1,600, that of the red cell solution 3·1 per cent. and the period of contact 2½ hours, the trypanocidal titre was 32 ; when, however, the concentration of tryparsamide was 1 : 100, that of the red cell solution 12·5 per cent. and the length of contact 6 hours, the titre obtained was 16,000. If a 1 per cent. solution of tryparsamide in 'nutrient medium' (equal parts of Ringer-glucose and rabbit serum) is incubated for 6 hours at 37° C., its trypanocidal titre is found to be about 10, as already mentioned. The trypanocidal power of tryparsamide under these conditions is, therefore, $100 \times 10 = 1{,}000$. When, however, the serum of the 'nutrient medium' is replaced by a 25 per cent. solution of rabbit red cells, the trypanocidal titre of the mixture is 16,000, so that under these conditions the trypanocidal power of tryparsamide is increased to $100 \times 16{,}000 = 1{,}600{,}000$.

In somewhat similar experiments intact rabbit red cells were used in place of the solution of laked red cells, a 1 : 50 solution of tryparsamide in Ringer-glucose being incubated for 6 hours at 37° C. with an equal volume of red blood corpuscles. The red cells were then separated from the Ringer-glucose-drug solution and the trypanocidal titres of the latter and of the laked corpuscles

determined. As will be seen from Table VI, the titre of the Ringer-glucose-drug solution was 8,000–16,000 (Specimen 3A) and that of the solution of red cells 4,000–8,000 (Specimen 3B). Apparently, therefore, intact red cells, like solutions of red cells, are capable of increasing greatly the trypanocidal power of tryparsamide.

A reasonable explanation of this phenomenon seems to be that some constituent of the intact red cells and also of the red cell solution has a capacity

TABLE VI

Comparison of the capacity of laked red cell solution and of intact red cells to activate tryparsamide

No. of Specimen	Specimen	No. of times specimen diluted	No. of living trypanosomes per 256 squares of the haemocytometer scale: Start	After 24 hours at 37° C.
1	Tryparsamide 1/50 in Ringer-glucose + equal volume rabbit serum Kept at 37° C. for 6 hours	4 8 16 32 64	86	0 0 9 64 80
2	Tryparsamide 1/50 in Ringer-glucose + equal volume 25 per cent. laked red cell solution Kept at 37° C. for 6 hours	4,096 8,192 16,384 32,768 65,536		0 0 0 50 68
3	Tryparsamide 1/50 in Ringer-glucose + equal volume red cells Kept at 37° C. for 6 hours and then the supernatant fluid separated from the red cells by centrifugation A. supernatant fluid	2,048 4,096 8,192 16,384 32,768		0 0 0 25 66
	B. red cells washed in iced saline, and then laked	1,024 2,048 4,096 8,192 16,384		0 0 0 30 80

of converting to some extent the relatively inert pentavalent tryparsamide into the highly trypanocidal corresponding trivalent compound, reduced tryparsamide.

CONSTITUENT OF ERYTHROCYTES RESPONSIBLE FOR ACTIVATION OF TRYPARSAMIDE

We have now to inquire what is the constituent of the red cell which is capable of so greatly increasing the trypanocidal power of solutions of a pentavalent arsenical, and in particular whether haemoglobin plays any rôle in the process.

The first point which we decided to investigate was whether the reaction was in any way modified according to whether the haemoglobin was in the form of oxyhaemoglobin, reduced haemoglobin or carboxyhaemoglobin. With this object in view, a 25 per cent. red cell solution was prepared and mixed with an equal volume of a solution of 2 per cent. tryparsamide. The mixture was then divided into three equal portions ; in the first the haemoglobin was maintained as oxyhaemoglobin by slowly bubbling oxygen through it, in the second as reduced haemoglobin by means of saturation with carbon dioxide, and in the third as carboxyhaemoglobin by passing through it a current of carbon monoxide. After these various mixtures had been kept under the above conditions for 6 hours at 37° C., their trypanocidal titres were determined. It was found that in each case the titre was 8,000–16,000, whereas that of a control, consisting of equal volumes of a 2 per cent. solution of tryparsamide and of normal rabbit's serum, was only 8. The fact that a solution of red cells activates tryparsamide to an equal extent, whether the haemoglobin is present in the form of oxyhaemoglobin, reduced haemoglobin or carboxyhaemoglobin, suggests that haemoglobin is not the constituent of the red cell responsible for the activation of the inert pentavalent arsenical compound.

Experiments were next conducted with a view to obtaining information on the effect of heat on the activating power of a solution of red cells. With this object in view, a 25 per cent. solution of red blood cells was made and divided into four equal portions. These were treated as follows :—Specimen A unheated ; Specimens B, C and D heated respectively to 56° C., 65° C. and 75° C. for half an hour. The effect of the procedure was :—in Specimen B the colour was slightly altered and there was a slight precipitate ; in Specimen C the colour was definitely altered and there was a considerable precipitate ; whereas in Specimen D all the haemoglobin was converted into a chocolate precipitate and a pale straw-coloured fluid left. After removing the deposits by centrifugation, an equal volume of Ringer-glucose solution, containing 1 : 50 tryparsamide, was added to each supernatant fluid, and the mixtures incubated at 37° C. for 6 hours, after which their trypanocidal titres were determined with the following results :

Specimen A (unheated) : 8,000–16,000.
,, B (heated to 56° C. for 30 minutes): 8,000.
,, C (,, 65° C. ,, ,,): 4,000.
,, D (,, 75° C. ,, ,,): 256.

From this it appears that heating to 65° C. for 30 minutes profoundly alters the haemoglobin but has little effect upon the activating capacity of the solution, whilst heating to 75° C. for 30 minutes completely precipitates the haemoglobin and greatly reduces the activating power of the solution. This work, therefore, affords additional evidence that haemoglobin is not responsible for the activating capacity of a solution of red cells, but that the phenomenon

is dependent on the presence of some other constituent which is relatively thermostable in that it withstands a temperature of 65° C. for 30 minutes, and is only partially destroyed by a temperature of 75° C. for 30 minutes.

In order still further to examine the question whether haemoglobin was in any way responsible for the capacity of a red blood cell solution to increase the trypanocidal power of a solution of tryparsamide, it was decided to determine the effect of incubating the drug in a haemoglobin solution prepared from the crystalline substance in the following manner :—

A litre of washed red corpuscles from a horse was laked in an equal volume of distilled water. The solution was then cooled to 0° C. and a litre of ice-cold ether was added. This mixture was kept at 0° C. for some hours, during which period it was frequently shaken. It was then centrifuged and the lowest layer, consisting of a clear solution of haemoglobin, withdrawn by means of a siphon. This solution was freed from ether by a current of air purified by passage through potassium permanganate and sulphuric acid. During the process the temperature was maintained at 0° C. A quarter volume of ice-cold alcohol was then added to the ether-free solution of haemoglobin and the mixture allowed to stand overnight in the refrigerator. The following morning the crystals of haemoglobin were removed by centrifugation, washed with ice-cold 20 per cent. alcohol and then with water at 0° C. They were then dissolved in distilled water at 30° C. and allowed to recrystallize in the refrigerator at −3° C. This process was repeated several times. (Plimmer, 1920.)

The purified crystals of haemoglobin thus prepared were dissolved in physiological saline, and the strength of the solution was adjusted by means of the colorimeter and spectroscope to equal a 10 per cent. solution of washed red cells from a normal rabbit. To equal volumes of these matched solutions of crystalline haemoglobin and of normal rabbit red cells were added equal volumes of a 1 : 50 solution of tryparsamide in Ringer-glucose. The mixtures were then incubated at 37° C. for 6 hours, and their trypanocidal titres determined in the usual manner. The trypanocidal titre of the mixture made from the laked red cell solution was 2,000–4,000, whereas that made from the crystalline haemoglobin solution was only 8 ; in other words, when tryparsamide is dissolved in crystalline haemoglobin solution, it exhibits no greater trypanocidal activity than when dissolved in Ringer-glucose solution or 'nutrient medium.' This experiment seems to afford conclusive evidence that haemoglobin *per se* is not responsible for the activation of tryparsamide.

During the course of our experiments on the activation of tryparsamide by solutions of red blood cells, we observed that solutions which had been kept for some time appeared to be less active than fresh solutions. The point was specifically examined in the following experiment :—

Experiment. Various 25 per cent. solutions of rabbit red cells made on different dates were stored at 0° C. From time to time equal volumes of these solutions were mixed with equal volumes of a 2 per cent. solution of tryparsamide in Ringer-glucose and the mixtures incubated at 37° C. for 6 hours. The trypanocidal titres of the mixtures were then determined in the usual manner, with the results shown in Table VII.

This work shows clearly that the storing of solutions of rabbit red cells at 0° C. did actually result in a loss of their power to activate tryparsamide.

The rate with which this power was lost apparently varied in the case of different red cells. Most of the solutions had lost within a period of 3 to 4 weeks almost the whole of their power to activate tryparsamide, and within about 6 or 8 weeks all solutions of red cells had become practically inert.

It is interesting to note that the haemoglobin values of these various solutions of red cells, as judged by the colorimeter and comparison spectroscope, had deteriorated but slightly during their prolonged sojourn in the refrigerator.

Table VII

Showing the effect of storing solutions of red cells at 0° C. upon their capacity to activate tryparsamide

Dates of determination of trypanocidal titres	Trypanocidal titres of mixtures of equal parts of a freshly-made 2 per cent. solution of tryparsamide and of various 25 per cent. solutions of red cells incubated at 37° C. for 6 hours — 25 per cent. red cell solutions made						Trypanocidal titre of a freshly-made 1 per cent. solution of tryparsamide in nutrient medium
	5 April	10 April	15 April	17 April	2 May	23 May	
5 April	8/16,000						8
10 ,,	4/8,000	8/16,000					8/16
15 ,,	4,000	4,000	16,000				8/16
17 ,,	2,000	1,000		8,000			8/16
2 May	2,000	64/128	8,000	1,000	8,000		8
23 ,,	64/128	64	256/512	64	64	16,000	8/16
30 ,,	32	16/32	128	64	16/32	8/16,000	8/16
25 June	32	32	32	32	32	256	8/16

SUMMARY AND DISCUSSION

It was found that, if red cells were suspended at 37° C. in a solution of reduced tryparsamide in either Ringer-glucose or ' nutrient medium,' a certain amount of the drug rapidly passed into the red cells. This was evident from the fact that when these red cells were laked, after separation from the drug solution and washing rapidly in large volumes of iced saline, the laked solution was powerfully trypanocidal. Furthermore, when drug-laden red cells, washed in iced saline, were subsequently suspended in Ringer-glucose or ' medium,' drug diffused out of the red cells into the surrounding fluid.

The amount of reduced tryparsamide which diffused into red cells depended firstly on the concentration of the drug in the surrounding medium, and secondly, although to a much less extent, on the length of time the red cells were exposed to the solution of drug. When red cells were suspended for 15 minutes at 37° C. in an equal volume of Ringer-glucose-drug solution containing 1 : 25,000 reduced tryparsamide, the concentration of drug within them was found to be 1/4th to 1/8th of that in the surrounding fluid ; when the concentration of the drug was increased 16 times, i.e., to 1 : 1,562·5, the concentration found in the red cells was about 1/16th of that of the surrounding fluid. The amount of

drug which had diffused into the red cells within 24 hours was in each case about double that found within 15 minutes. It was immaterial whether the drug was dissolved in Ringer-glucose alone or in ' nutrient medium ' (equal parts of Ringer-glucose solution and rabbit serum heated to 64° C. for 30 minutes).

From red cells which had been in contact with the drug solution for 15 minutes, and then subsequently washed in iced saline, a large proportion of the drug which they contained readily diffused out when they were suspended in drug-free Ringer-glucose solution or ' nutrient medium ' at 37° C. When red cells which had been in contact with drug solution for 24 hours were treated in a similar manner, the amount of drug which diffused out into the normal Ringer-glucose solution or ' nutrient medium ' was definitely less, notwithstanding the fact that laked solutions of such red cells showed that they actually contained about twice as much drug as did those which had been exposed to the drug solution for only 15 minutes. This is an interesting fact and suggests that, after prolonged sojourn of red cells in drug solution, the drug becomes in some way more firmly bound to the substance of the red cells, so that it is unable to diffuse out into drug-free Ringer-glucose or ' medium,' but that it is not so firmly bound as to prevent the laked solution manifesting its full trypanocidal activity.

Another interesting point which emerges from this work is that, whereas both 1 : 25,000 and 1 : 1,562·5 solutions of reduced tryparsamide in Ringer-glucose preserve practically completely their trypanocidal power after storing at 37° C. for 24 hours, the weaker concentration of drug in ' nutrient medium ' loses over 90 per cent. of its trypanocidal activity. This suggests that the drug in some way combines with the protein of the ' medium ' to form an inert substance. The addition of an equal volume of red cells to a 1 : 25,000 solution of reduced tryparsamide in ' medium ' suffices, for some reason for which we have no explanation, to inhibit this loss of trypanocidal power.

Similar experiments on the diffusibility into red cells of the pentavalent compound tryparsamide gave more complicated results. Whilst there seemed to be no doubt that tryparsamide, like its reduced homologue, diffused readily into red cells, it became at once obvious that another factor was at work which largely obscured the main issue. When red cells, which had been in contact for some hours with 0·5 per cent. solution of tryparsamide, and then washed thoroughly in iced saline, were suspended in drug-free Ringer-glucose solution, a substance was found to have diffused out of the red cells which was of enormously greater trypanocidal power than the 0·5 per cent. solution of tryparsamide. It is, therefore, clear that red cells are, in some way, able to change the relatively inert tryparsamide into a highly trypanocidal substance.

This recalls certain interesting observations made many years ago by Levaditi, Yamanouchi and others. Levaditi and Yamanouchi (1908) showed that emulsions of liver, muscle and lung incubated with atoxyl transformed

it into a trypanocidal substance which they termed ' trypanotoxyl.' Levaditi (1909) believed that the transforming agent was found in the liver and certain other organs, and concluded that the active substance was a reduction product of the drug combined with protein to form an arseniated toxalbumen. Yamanouchi (1910) considered that the trypanocidal substance was produced by the red cells ; he found that liver and other organs cleared of blood no longer possessed the power of activating atoxyl. Yamanouchi further observed that red cells acted more powerfully in the presence of carbon dioxide than under normal conditions, and that in the presence of oxygen they failed altogether to activate atoxyl ; pure recrystallized haemoglobin was without action. The active substance was soluble in alcohol, thermostable, and free from protein material. Terry (1912) found that both liver and blood, when incubated with atoxyl, transformed the drug into a toxic substance. The transforming agent in liver had, however, characteristics which, in some respects, were quite different from those of the active agent in blood. The active agent in liver was soluble in salt solution, filtrable through collodion, and resisted 100° C. for 10 minutes ; the active agent in blood was not extractable by salt solution, was very easily destroyed, losing practically all its activity if the blood were laked, and was completely destroyed by heating to 100° C. for 10 minutes. In a later paper (1915), Terry showed that the toxic substance into which atoxyl is transformed (transformed atoxyl) is thermostable, but that the transforming agent in blood is thermolabile.

While we have not attempted to examine the points at issue between Levaditi and Yamanouchi, or to investigate the exact nature of the active substance which is produced when blood is allowed to act upon a pentavalent arsenical, e.g., whether it is a simple reduction product or something in the nature of what Levaditi described as an arseniated toxalbumen, we have concerned ourselves with a preliminary inquiry regarding the constituent of the red cell which is capable of increasing the trypanocidal power of pentavalent arsenicals.

Our experiments showed that a solution of laked red cells was also able to activate tryparsamide in a similar degree to intact red cells. The extent to which a solution of red cells can activate tryparsamide is exceedingly great, as is shown by the following observation. The trypanocidal titre of a 1 per cent. solution of tryparsamide in ' medium,' either freshly-made or kept for 6 hours at 37° C., is 8 ; the trypanocidal titre of a 1 per cent. solution of tryparsamide in a 12·5 per cent. solution of red cells which has been kept for 6 hours at 37° C. is about 16,000 ; thus, by substituting the red cell solution for ' medium,' the trypanocidal titre is increased no less than 2,000 times.

We do not know what constituent of the blood is responsible for producing this change. It cannot be haemoglobin itself since solutions prepared from pure crystalline haemoglobin showed no power to activate tryparsamide ; and, furthermore, no differences were observed whether the haemoglobin was in the form of oxyhaemoglobin, reduced haemoglobin or carboxyhaemoglobin.

Whatever its nature, the activating substance is relatively thermostable in that it resists almost completely a temperature of 65° C. for 30 minutes, and is not completely destroyed by a temperature of 75° C. for 30 minutes. The activating power of red cell solutions kept at 0° C. is gradually lost, so that, within two months or less, such solutions have become practically inert.

REFERENCES

LEVADITI, C. (1909*a*). Mécanisme d'action des composés arsenicaux dans les trypanosomiases. *Bull. Soc. Path. Exot.*, **2**, 45.

——— (1909*b*). Le mécanisme d'action des dérivés arsenicaux dans les trypanosomiases. *Ann. Inst. Pasteur*, **23**, 604.

——— and YAMANOUCHI, T. (1908). Mécanisme d'action de l'atoxyl dans les trypanosomiases. *C.R. Soc. Biol.*, **65**, 23.

MURGATROYD, F., RUSSELL, HELEN, and YORKE, W. (1934). Studies in Chemotherapy. XI: The trypanocidal titre of the serum of rabbits after the intravenous injection of various compounds of arsenic. *Ann. Trop. Med. & Parasitol.*, **28**, 227.

PLIMMER, R. H. A. (1920). Practical organic and bio-chemistry. Longmans, Green and Co., Lond.

TERRY, B. T. (1912). The action of atoxyl. *Proc. Soc. Exper. Biol. & Med.*, **9**, 41.

——— (1915*a*). Different amounts of transformed atoxyl produced by incubating 1 per cent. and 10 per cent. atoxyl in blood. *Jl. Exper. Med.*, **21**, 258.

——— (1915*b*). The effect of heat on the transforming and binding power of blood. *Ibid.*, **21**, 267.

YAMANOUCHI, T. (1910). Action de l'atoxyl sur les trypanosomiases dans l'organisme. *C.R. Soc. Biol.*, **68**, 120.

YORKE, W., and MURGATROYD, F. (1930). Studies in Chemotherapy. III: The action *in vitro* of certain arsenical and antimonial compounds on *T. rhodesiense* and on atoxyl- and acriflavine-resistant strains of this parasite. *Ann. Trop. Med. & Parasitol.*, **24**, 449.

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